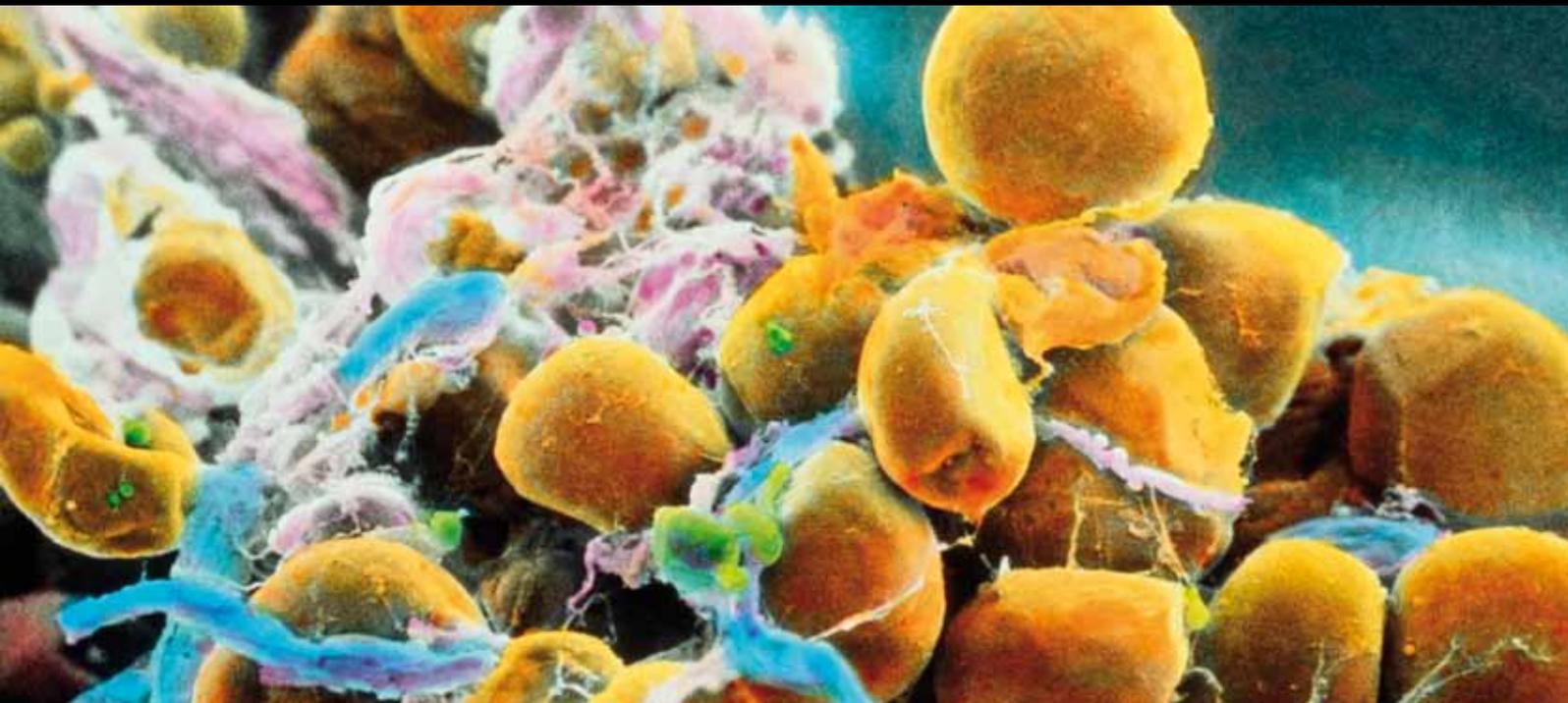


MANIFESTATIONS of Adipose TISSUE DYSFUNCTION

GUEST EDITORS: NICOLA ABATE, ANNE E. SUMNER, AND MANISHA S. CHANDALIA





Manifestations of Adipose Tissue Dysfunction

Journal of Obesity

Manifestations of Adipose Tissue Dysfunction

Guest Editors: Nicola Abate, Anne E. Sumner,
and Manisha S. Chandalia



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Editorial

Manifestations of Adipose Tissue Dysfunction

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Adipose tissue is regarded as an endocrine organ which contributes to regulating systemic energy balance. Since adipocyte is the specialized cell that can safely store triglyceride, normal adipose tissue function in buffering excessive caloric intake is able to prevent systemic metabolic consequences related to high glucose and lipid content in nonadipose cells. Excessive caloric intake relative to energy expenditure is rapidly becoming a feature of most populations around the world, and inability of adipose tissue to fulfill its role is inevitably linked to metabolic abnormalities recognized to increase risk for cardiovascular disease (CVD) and type 2 diabetes (T2D). Several investigators currently advocate focusing on improving our knowledge of mechanisms regulating adipose tissue function independent of adipose tissue mass. Filling this gap in the literature will better enable us to identify novel approaches to treat individuals at risk or with established T2DM and CVD. This special issue is dedicated to various manifestations of adipose tissue dysfunction and, with a combination of clinical papers and reviews of the literature, it addresses various aspects of recent advances and opportunities in this field of research.

The various papers discuss the existing evidence regarding the role of functional heterogeneity of various adipose tissue areas in determining risk for metabolic complications, such as insulin resistance and dyslipidemia. There is a discussion on recent evidence for adipose tissue inflammation and adipokines production as determinants of systemic metabolic abnormalities of lipid and glucose metabolism. The overall theme is completed by the topics of potential directions for future research and evaluation of the role that maternal

nutrition may have in the early development of adipose tissue dysfunction.

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Clinical Study

Waist Circumference as Measure of Abdominal Fat Compartments

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This study examines intercorrelations among waist circumference (WC), intraperitoneal fat (IPF), and subcutaneous abdominal fat (SAF) in ethnically diverse Dallas Heart Study consisting of 1538 women and 1212 men (50% Black). Correlations between fat depots and triglyceride or HOMA2-IR, biomarkers of metabolic syndrome, are also reported. Total abdominal fat (TAF), ASF, and IPF masses were measured by magnetic resonance imaging. The highest correlations with WC according to ethnicity and gender were noted for TAF ($R^2 = 0.81 - 0.88$) with progressively lower correlations with ASF (0.65–0.82) and IPF (0.29–0.85). The percentage of IPF relative to TAF was not significantly correlated with WC. For all WC categories, higher IPF/ASF ratios were associated with higher triglyceride levels. In contrast, differences in ratios had little or no association with HOMA2-IR. However, when all data were pooled, IPF was positively correlated with both triglyceride ($r = 0.358$ (men) and 0.363 (women)) and HOMA2-IR ($r = 0.480$ (men) and 0.517 (women)); after adjustment for ASF, IPF was still correlated with triglyceride ($r = 0.353$ (men) and 0.348 (women)) and HOMA2-IR ($r = 0.290$ (men) and 0.221 (women)). WC measures TAF reliably, but its association with IPF depends on IPF/ASF ratios that vary by gender and ethnicity.

1. Introduction

Abdominal obesity is one component of the metabolic syndrome [1]. Clinically, abdominal obesity is identified by an increase in waist circumference (WC). Increased WC has repeatedly been linked to metabolic risk. It is unclear, however, whether this measure is a correlate of increased risk through its correlation with total abdominal fat (TAF) or a specific, metabolically unhealthy depot of adipose tissue. Many investigators postulate that the key component of body fat underlying the metabolic syndrome is intraperitoneal fat (IPF) or visceral fat [2–7]. Others nonetheless contend that abdominal subcutaneous fat (ASF) is a more important pathogenic factor [8–14]. Since previous studies have shown that IPF and ASF are intercorrelated [15], the more important adipose-tissue compartment underlying the metabolic syndrome is difficult to identify.

The primary aim of this study was to determine the strength of the correlations between WC and TAF, and ASF and IPF measured by magnetic resonance imaging (MRI). These analyses were made for gender in whites, blacks, and Hispanics of the Dallas Heart Study [16]. We additionally correlated SAF and IPF with plasma triglyceride (TG) and homeostatic model assessment of insulin resistance (HOMA2-IR) [17], both accompanying the metabolic syndrome.

2. Methods

Details of DHS study recruitment have been published previously [16]. The current cohort consisted of 1538 women (50% black, 29% white, and 21% Hispanic) and 1212 men (50% black, 36% white, and 16% Hispanic) that had measurement of ASF, IPF, and retroperitoneal fat (RPF). DHS study participants of other ethnicities were excluded from the study. All

TABLE 1: Subject characteristics.

	Men			Women		
	Black	White	Hispanic	Black	White	Hispanic
Number of subjects	579	434	199	767	449	272
Age (years)	46 (10) ^a	45 (9) ^a	41 (9) ^a	45 (10)	46 (10)	41 (9) ^a
BMI (kg/m ²)	29.5 (6.8)	28.9 (5.5)	29.4 (4.6)	32.9 (8.3) ^a	28.6 (6.9) ^b	30.9 (7.4)
Waist circumference (cm)	101 (14)	103 (14) ^b	99 (11)	100.7 (17.2) ^d	91.4 (15.8)	94.5 (16.2)
Hip circumference (cm)	106 (14)	106 (11)	102 (8) ^a	115.6 (16.4) ^d	109.4 (14.9)	109.5 (15.3)
Glucose (mg/dL)	108 (52)	98 (29) ^c	104 (34)	103.5 (48.3)	95.4 (31.6) ^b	107.6 (48.5)
Insulin (pmol/L) median (IQ)	85 (103)	75 (82) ^b	91 (83)	106 (94)	68 (75) ^b	103 (100)
HOMA2-IR (%) median (IQ)	1.63 (1.93)	1.42 (1.51) ^b	1.72 (1.58)	3.63 (3.77)	2.23 (2.75) ^b	3.51 (3.60)
Triglycerides (mg/dL) median (IQ)	121 (120) ^d	157 (121)	170 (131)	80 (52) ^d	98 (75)	111 (77)
HDL cholesterol (mg/dL)	50 (15) ^d	42 (10)	42 (10)	54 (15)	55 (17)	49 (12) ^a
Non-HDL cholesterol (mg/dL)	128 (43) ^d	142 (39)	145 (41)	125 (41)	128 (38)	129 (38)
Systolic blood pressure (Hg mm)	132 (18) ^d	127 (13) ^c	124 (13)	120 (15) ^d	124 (16)	129 (19)
Diastolic blood pressure (Hg mm)	79 (10)	79 (9)	76 (9) ^a	80 (10) ^d	76 (9)	78 (9)
% Metabolic syndrome	30.0	33.9	35.0	42.4	31.5	39.3
% Diabetes mellitus	13.2	5.6	9.7	11.3	5.6	10.8

^aSignificantly different from Blacks and Whites; $P \leq 0.0002$; ^bsignificantly different from Blacks and Hispanics; $P \leq 0.03$; ^csignificantly different from Blacks; $P = 0.0001$; ^dsignificantly different from Whites and Hispanic; $P < 0.0001$; ^esignificantly different from Hispanics; $P < 0.0001$.

study volunteers gave written informed consent to participate in an Institutional Review-Board-approved study.

Body weight was measured with a portable scale (Ever Weigh, Lithium electronic scale no. 34067, Health O Meter, Bridgeview, IL, USA) to the nearest 0.1 kg. Height was measured with a stadiometer. Subjects were in a standing position with arms on side, legs straight, and knees together, with feet flat pointed outward. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest using a stretch-resistant tape with a spring providing constant tension. Fasting plasma lipids, glucose, and insulin were measured as described previously [16]. Insulin resistance was estimated with the HOMA2 computer model (HOMA Calculator version 2.2) [17]. Three categories of WC were defined for this study: low, intermediate, and high. Low WC corresponded to <90 cm in men and <80 cm in women; intermediate WCs were 90–101 cm in men and 80–89 cm in women; and high WCs were ≥ 102 cm in men and ≥ 90 cm in women. These cut points corresponded to DHS body mass index categories of <25 kg/m², 25–29.9 kg/m², and ≥ 30 kg/m². Zhu et al. [18] reported essentially the same ranges based on NHANES III data. Measurements of abdominal compartments of body fat were performed using 1.5 Tesla MRI scanners (Intera; Philips Medical Systems, Best, The Netherlands). The entire abdomen from the diaphragm to the pelvis was scanned using contiguous axial 10 mm slices, as previously described [19]. A single MRI slice at the L2-L3 level was used to quantify total abdominal fat (TAF), ASE, IPF, and retroperitoneal fat (RPF) as detailed by Abate et al. [19]. Briefly, the validation of this method to quantify total abdominal fat subregions involved MRI measurements from the 12th thoracic to 1st sacral vertebra calculated from contiguous 10 mm thick slices that covered the entire abdomen. Regression functions were derived that predicted total fat masses in the respective

compartments and that correlated best with the single slice measurement at L2-L3 level. Similar analyses were done to validate the measurement in women [15].

3. Statistics

Linear descriptive statistics were employed in data analyses. Data are summarized as means \pm S.D. or S.E. for metabolic parameters. For data not normally distributed, results are given as medians (with interquartiles), and data were log-transformed prior to parametric statistical comparisons. Comparisons of means of metabolic risk factors among ethnic groups within each gender were done for metabolic parameters using ANOVA with Bonferroni adjustments for multiplicity of testing or in selected cases using a posthoc Fisher *F* test. Pearson's correlation coefficients were determined for analyses of linear associations of waist girth to abdominal fat parameters measured by MRI. Spearman's and partial correlations were also calculated for relating adipose tissue compartments to triglycerides and HOMA2-IR. A SAS version of StatView (version 5.1.26) was employed for the analyses.

4. Results

The clinical characteristics of subjects according to ethnicity and gender are shown in Table 1. Mean ages were in the 40's. Mean BMIs ranged from 28.6 to 32.9 kg/m² for all groups. Mean WCs ranged from 99 cm to 101 cm for the three groups of men and from 91.4 cm to 100.7 in women; WCs were higher in Black women. In both men and women, Blacks had the lowest TG and Hispanics had the highest. Black men had higher mean HDL-C levels compared to Whites and Hispanics; differences among the three groups of women were less.

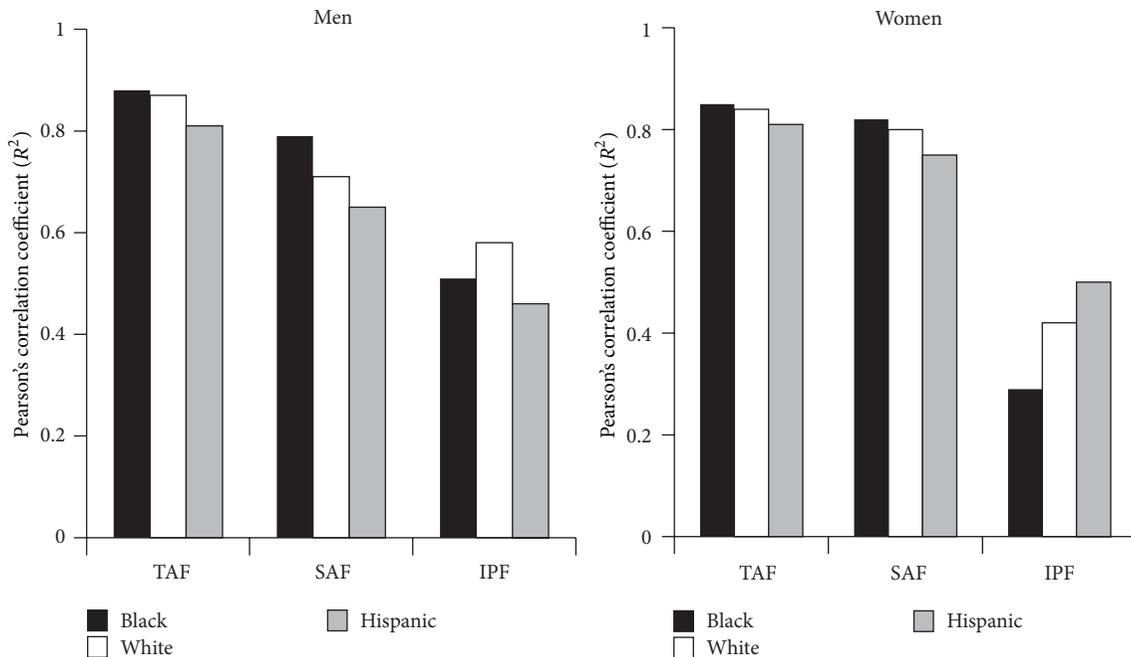


FIGURE 1: Pearson's correlation coefficients (r^2) by ethnicity and gender for linear regression analyses of waist circumference versus total abdominal fat (TAF), subcutaneous abdominal fat (SAF), and intraperitoneal fat (IPF). Highest correlations were found for TAF, intermediate for SAF, and lowest for IPF. All correlations were significant at $P < 0.001$.

Black men had lower non-HDL-C levels than Whites and Hispanics, but they had higher systolic blood pressures.

Pearson's correlations (R^2) for linear regression analyses between WCs and different abdominal-fat compartments are shown in Figure 1. The highest coefficients of correlation were noted for TAF with progressively lower correlation coefficient with ASF and IPF. IPF was better correlated with WC in men than in women, but still, WC was not a good indicator of IPF. The strength of the correlations was similar within each ethnic group at a $P < 0.0001$.

Absolute fat masses in IPF and ASF for three categories of WC—low, intermediate, and high—according to ethnicity and gender are presented in Figures 2(a) and 2(b), respectively. Women of all ethnicities had much lower IPF masses than men. Further, in both men and women, blacks had lower IPF masses than whites and Hispanics at all levels of WC ($P < 0.02$); white men with low and intermediate WC had lower IPF than Hispanics ($P < 0.02$); and white women at intermediate WC had lower IPF than Hispanic women ($P < 0.02$). In contrast, men and women had similar patterns of ASF masses for each waist circumference category. Black men had significantly lower ASF than white and Hispanic for those with a low waist circumference, and they also had the highest ASF for those in the highest waist circumference category. The same pattern of ASF fat was noted in black women.

Figure 3 displays the IPF as a percentage of TAF. In all ethnicities, higher WCs matched with greater fat masses in each compartment (Figures 2(a) and 2(b)). Although fat masses rose with increasing WCs, the percentage of IPF relative to TAF did not rise for low, intermediate (MED), and high WCs for men or women (Figure 3). In general, blacks had slightly lower percentages of IPF than both other groups.

In Figures 4(a) and 4(b), ranges of IPF masses are given for quintiles of TAF in men and women, respectively. As TAF rose, so did IPF, showing that most of IPF mass was determined by TAF content. Within each TAF category, nonetheless, there was a range of IPF masses. This range broadened with higher TAF masses, suggesting heterogeneity of IPF response to obesity. In the most obese subjects, IPF masses varied over extremes of about 1.5 kg in men and 1.0 kg in women.

The means and distributions of the IPF/ASF ratio are shown for men and women of the three ethnic groups (Table 2). The distributions were skewed so that mean and 50th percentiles (medians) are not identical. Although mean percentage IPF was relatively constant with increasing WCs, great individual variation was noted across the span of IPF/ASF ratios.

To examine whether differences in IPF/ASF ratios affect plasma TG or HOMA2-IR, ratios were split into upper and lower halves and were related to these metabolic measures. The results for men are given in Figure 5(a). On the whole, for the three WC categories, greater IPF/ASF ratios associated with higher TG levels. In contrast, differences in ratios had little or no influence on HOMA2-IR. In women, a similar but less pronounced trend was noted for differences in IPF/ASF ratios on TG levels (Figure 5(b)). Differences in ratios again had little or no effect on HOMA2-IR.

To determine whether a more sensitive analysis might identify an effect of IPF on HOMA2-IR, Spearman's correlation coefficients were determined on all men and all women, regardless of ethnicity or WC (Figure 6). In both men and women, ASF was less strongly correlated with TG than was IPF. After cross-adjustment, ASF lost its association with TG

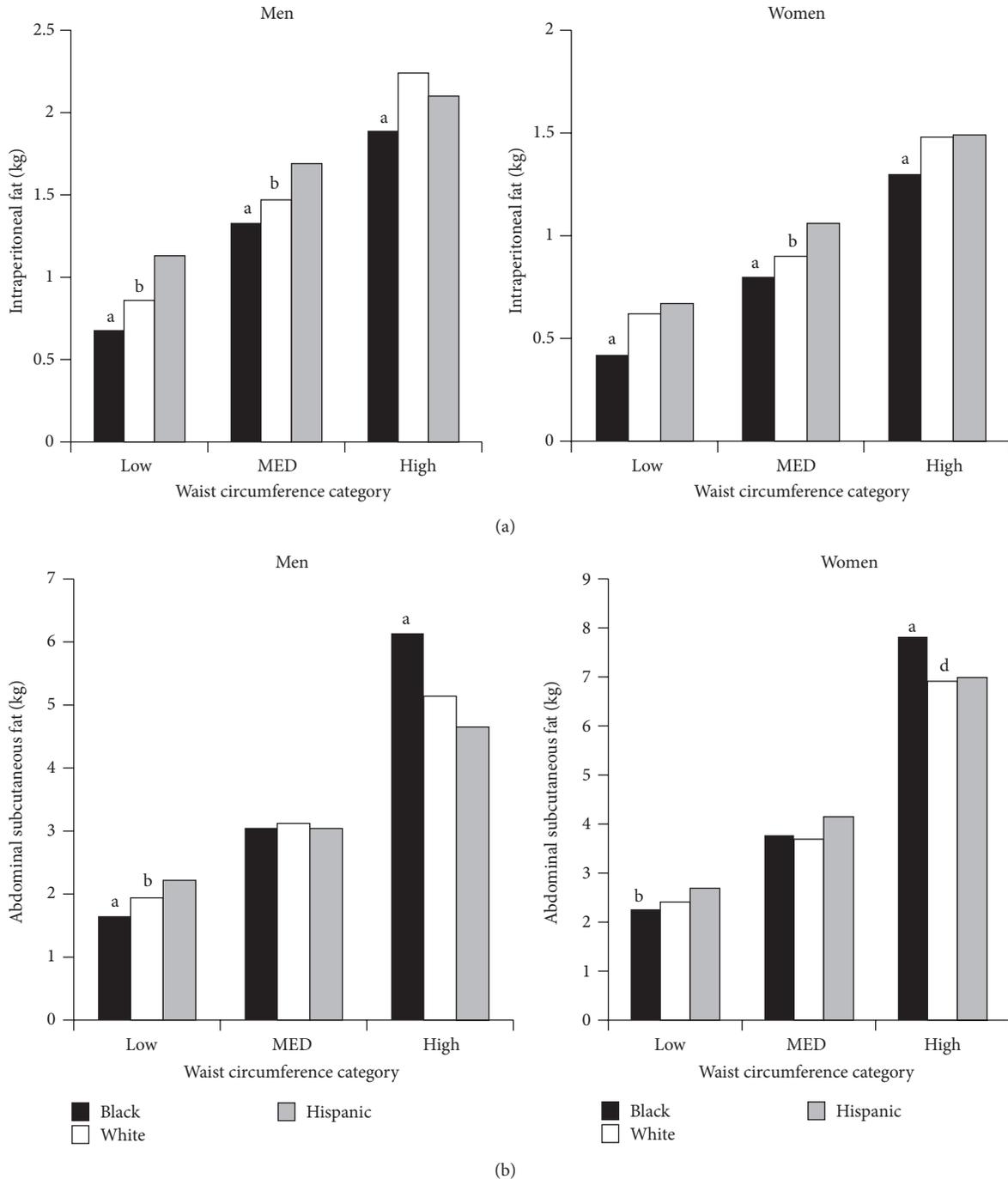


FIGURE 2: (a) Masses (kg) of intraperitoneal fat (IPF) for each waist circumference category for ethnicity and gender. Amounts of IPF increased for low, intermediate (MED), and high waist circumference categories. ^aSignificantly different from whites and Hispanics ($P < 0.02$); ^bSignificantly different from Hispanics ($P < 0.02$). (b) Masses (kg) of abdominal subcutaneous fat (ASF) for each waist circumference category for ethnicity and gender. Amounts of ASF increased for low, intermediate (MED), and high waist circumference categories. ^aSignificantly different from whites and Hispanics ($P < 0.02$); ^bsignificantly different from Hispanics ($P < 0.02$).

levels, whereas IPF did not. In both men and women, ASF and IPF were similarly correlated with HOMA2-IR. After cross-adjustment, the strength of the correlation for each compartment diminished, but IPF remained significantly correlated.

5. Discussion

The major findings of this study were the following. First, WC correlated strongly with TAF and ASF, whereas WC less strongly predicted IPF (Figure 1). Second, IPF constituted

TABLE 2: Distribution of intraperitoneal/abdominal subcutaneous fat ratio.

	Mean (SD)	10th	25th	50th	75th	90th
All men	0.462 (0.196)	0.247	0.323	0.426	0.568	0.731
Black	0.416 (0.184) ^a	0.222	0.286	0.387	0.505	0.662
White	0.488 (0.195)	0.262	0.353	0.462	0.592	0.759
Hispanic	0.541 (0.200)	0.318	0.405	0.498	0.655	0.837
All women	0.222 (0.091)	0.118	0.157	0.209	0.273	0.334
Black	0.198 (0.085) ^a	0.101	0.138	0.185	0.247	0.309
White	0.248 (0.090)	0.154	0.186	0.234	0.29	0.358
Hispanic	0.247 (0.092)	0.143	0.183	0.226	0.301	0.375

^aSignificantly different from White and Hispanic ($P < 0.0001$).

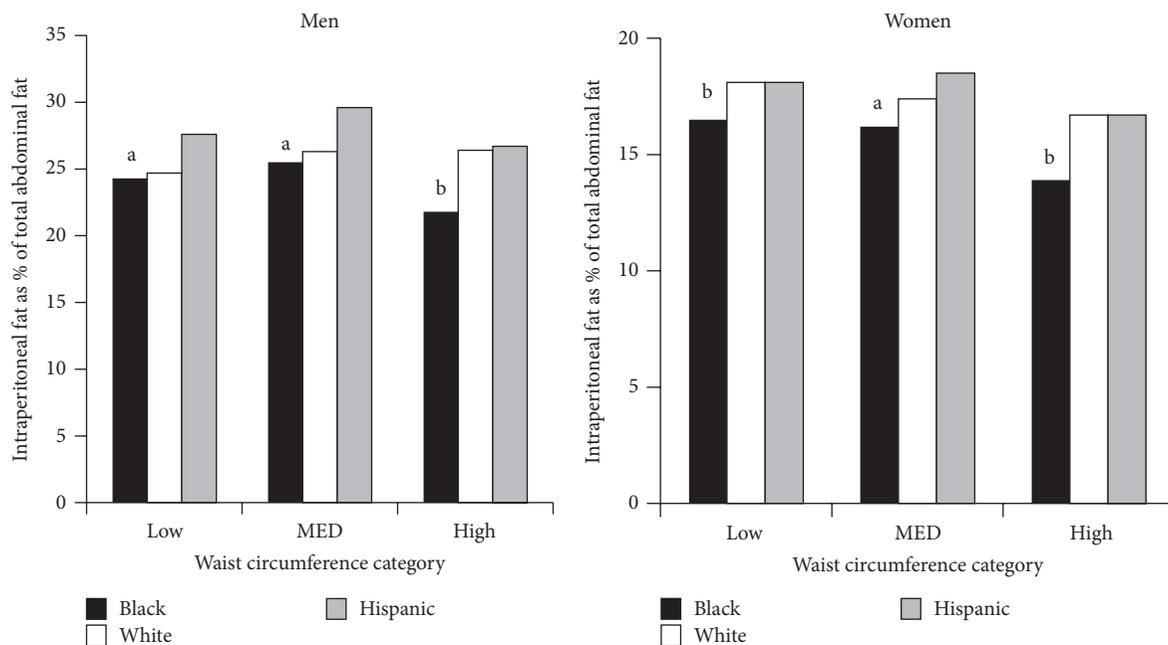


FIGURE 3: Percentage of intraperitoneal fat (IPF) or total abdominal fat (TAF) for each waist circumference category for ethnicity and gender. Blacks generally had a lower percentage IPF compared to whites and Hispanics. ^aSignificantly different from whites and Hispanics ($P < 0.02$); ^bsignificantly different from Hispanics ($P < 0.02$).

only about one-fourth of TAF in men and one-fifth in women (Figure 4). Third, for all groups, the distributions of IPF/ASF ratios showed considerable variability among individuals; this explains the relatively low correlations between WC and IPF (Table 2). Fourth, IPF rose progressively with increasing TAF, but at each step of increase, IPF masses varied considerably (Figures 4(a) and 4(b)). Fifth, for all WC categories, persons with higher IPF/ASF ratios had higher plasma TG levels than did those with lower ratios; this relationship was not observed for HOMA2-IR (Figures 5(a) and 5(b)). Even so when all ethnic groups were combined, a positive correlation was uncovered between IPF and HOMA2-IR, which persisted after adjustment for ASF (Figure 6).

IPF, ASF, and the distribution of their ratios were compared to blacks, whites, and Hispanic men and women. Both black men and women had lower median IPF/ASF ratios compared to whites and Hispanics (Table 2). In the three ethnic groups, TAF, ASF, and IPF were similarly correlated

with waist girths (Figure 1). In low, intermediate, and high WC categories, both black men and women had lower masses of IPF, compared to whites and Hispanics (Figure 2(a)). In low and intermediate WC categories of men, Hispanics had greater masses of IPF than whites as well as black; at high WG, only black men were different from the other ethnicities. In women, only blacks were consistently different from whites and Hispanics in IPF masses (Figure 2(a)). Black men and women had relatively low ASF mass compared to whites and Hispanics; but in the high WG categories, blacks of both genders had higher ASF mass than whites and Hispanics (Figure 2(b)).

A high WC clearly associates with all metabolic risk factors [1]; and it is commonly believed that WC is a surrogate measurement for visceral adipose tissue [20–22]. The current study revealed that amounts of IPF increased progressively through each category of increasing WC. In this sense, therefore, it can be said that WC is a surrogate for IPF.

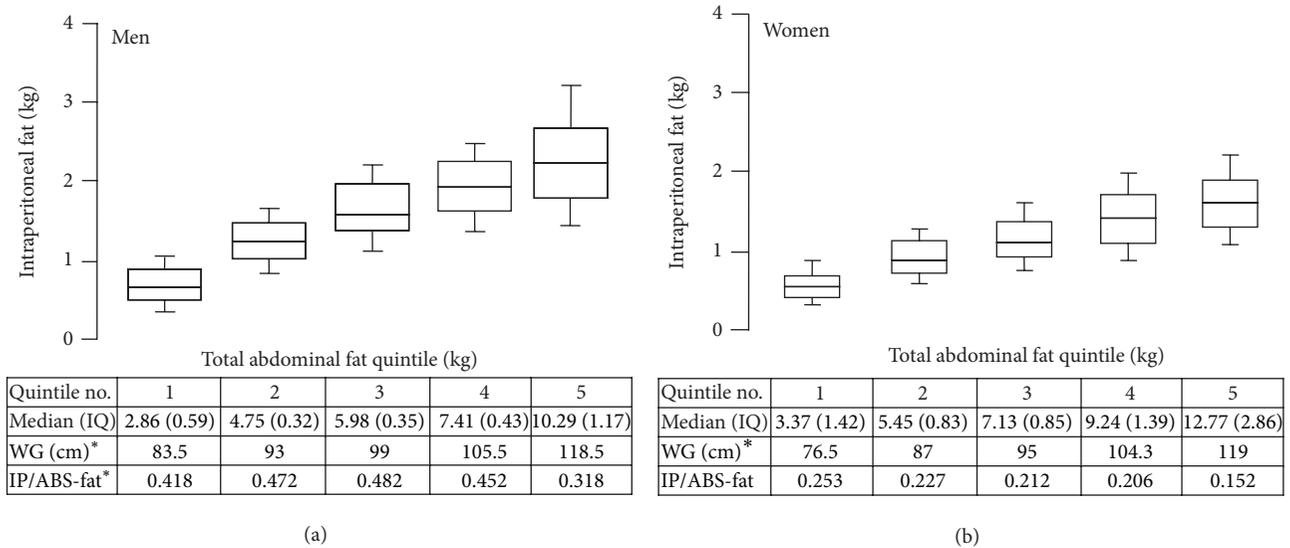


FIGURE 4: (a) Masses (kg) of intraperitoneal fat (IPF) plotted against masses of quintiles of total abdominal fat for all men. IPF masses increased progressively with TAF, and the distribution of IPF for each category widened. Boxes show mean and one standard deviation; whiskers show 2 standard deviations. The chart gives median values for each quintile, the mean waist girth, and ratio of IPF to abdominal subcutaneous fat. The latter ratio changed a little across ratios except in the highest quintile. (b) Masses (kg) of intraperitoneal fat (IPF) plotted against masses of quintiles of total abdominal fat for all women. IPF masses increased progressively with TAF, and the distribution of IPF for each category widened. Boxes show mean and one standard deviation; whiskers show 2 standard deviations. The chart gives median values for each quintile, the mean waist girth, and ratio of IPF to abdominal subcutaneous fat. The latter ratio changed a little across ratios except in the highest quintile.

The current data, nonetheless, indicate that WC is much more strongly correlated with TAF and ASF than with IPF. This being the case, it cannot be assumed that the relation between increased WC and metabolic syndrome is mediated predominantly through a higher IPF.

Abdominal obesity is well recognized to predispose to hypertriglyceridemia [23]. Our study found clear evidence that IPF correlates with plasma TG. For all WC categories and in men and women, those with higher IPF/ASF ratios had higher plasma TG levels. In addition, partial correlation analysis indicated that IPF independently associates with TG levels. The mechanism for this relationship can be readily visualized. Since IPF drains its fatty acids directly into the splanchnic circulation, these fatty acids should add an excess load of lipid on the liver beyond what would be derived from subcutaneous adipose tissue beds. This extra load should translate into higher TG levels.

Several reports suggest that IPF is related to insulin resistance. For instance, Carr et al. [2] reported that intra-abdominal fat is independently associated with insulin resistance, and others found a similar relationship [12, 24, 25]. It might be expected that if IPF causes insulin resistance, a high level of IPF should be a risk factor for type 2 diabetes. Such has been reported [6, 25, 26]. In the current study, IPF appeared to be correlated with HOMA2-IR, albeit weakly. This relationship could not be found when people with high and low IPF/ASF ratios were compared. But partial correlations suggest that higher levels of IPF associate with increased HOMA2-IR independently of ASF. In the light of previous reports, there seems to be little doubt that a positive correlation between IPF and insulin resistance exists.

IPF could be related to either insulin resistance in skeletal muscle or liver. The mechanisms whereby IPF per se could cause skeletal muscle insulin resistance are not readily apparent. An increased release of fatty acids from IPF is one possibility; but amounts must be relatively small compared to the total adipose tissue output of fatty acids. It is thus unlikely that a relatively small increment in release of fatty acids from IPF could substantially worsen insulin resistance in skeletal muscle [27–30].

If IPF increases insulin resistance, it is more likely to be hepatic insulin resistance; this condition is characterized by increased hepatic glucose output. Presumably an increased fatty acid influx into the liver suppresses insulin action, stimulates gluconeogenesis, and raises hepatic glucose output [31, 32]. A report suggests that HOMA2-IR reflects hepatic glucose output more than skeletal muscle insulin resistance [33].

The positive correlation between IPF and HOMA2-IR thus could be mediated through fatty acid stimulation of hepatic glucose output. An interesting question is what are the sources of fatty acids reaching the liver? This question has been examined by Nielsen et al. [28]. They found that the contribution of IPF to hepatic fatty acid delivery ranged from <10% to approximately 50% depending on amounts of IPF. The remainder of fatty acid flux to liver derived from subcutaneous adipose tissue. Thus their findings suggest that excess fatty acids from IPF could drive gluconeogenesis and raise HOMA2-IR.

In reference to the association between visceral obesity and metabolic risk factors, it seems important to distinguish between the rise in IPF with total body obesity and the

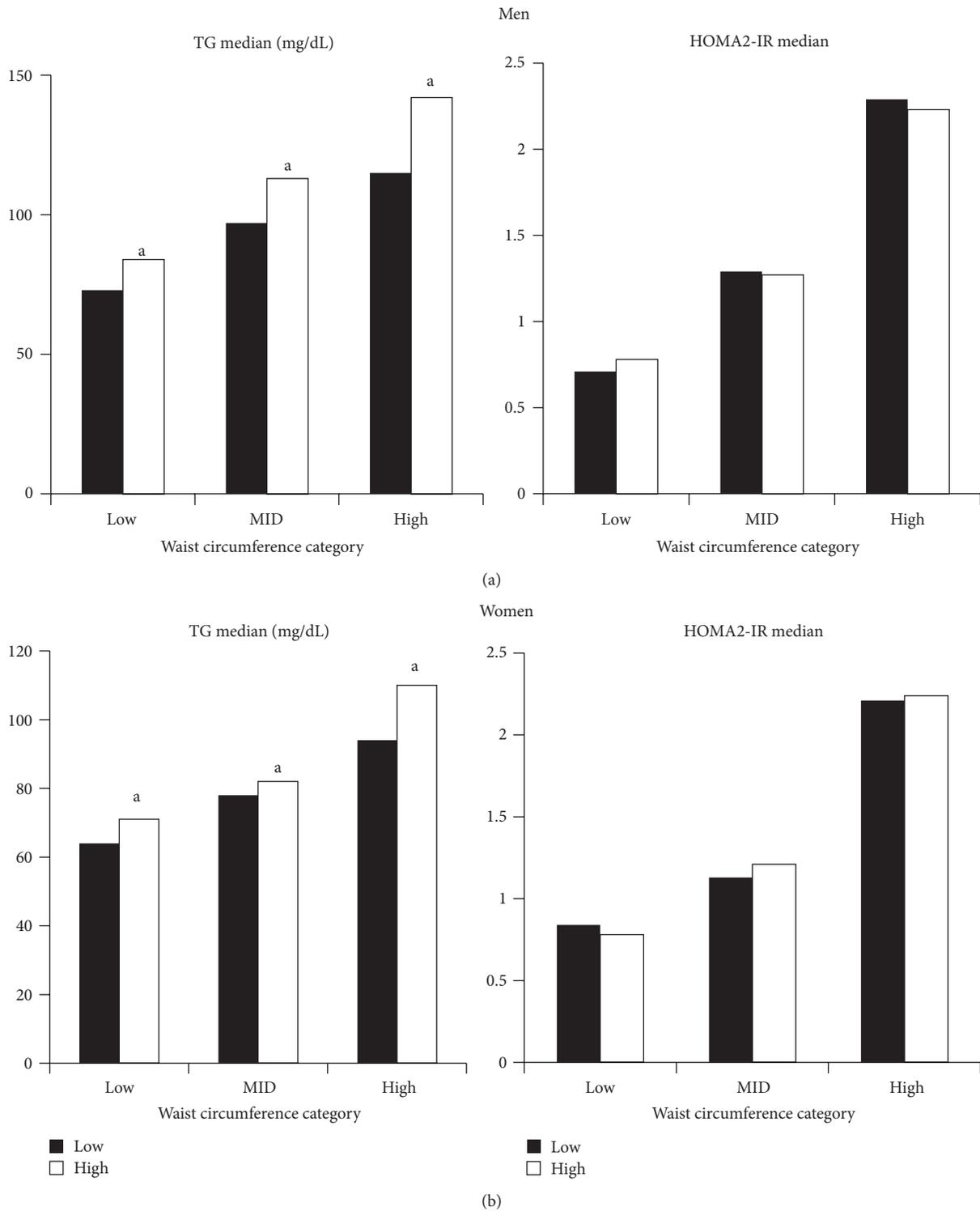


FIGURE 5: (a) Plasma triglyceride (TG) and HOMA2-IR for upper and lower halves of the intraperitoneal fat/abdominal subcutaneous fat ratios for all men. For TG, those with the high ratios had significantly higher triglyceride in each waist circumference category (^a $P < 0.05$). For HOMA2-IR, there were no differences between higher and lower ratios. (b) Plasma triglyceride (TG) and HOMA2-IR for upper and lower halves of the intraperitoneal fat/abdominal subcutaneous fat ratios for all women. For TG, those with the high ratios had significantly higher triglyceride in each waist circumference category (^a $P < 0.05$). For HOMA2-IR, there were no differences between higher and lower ratios.

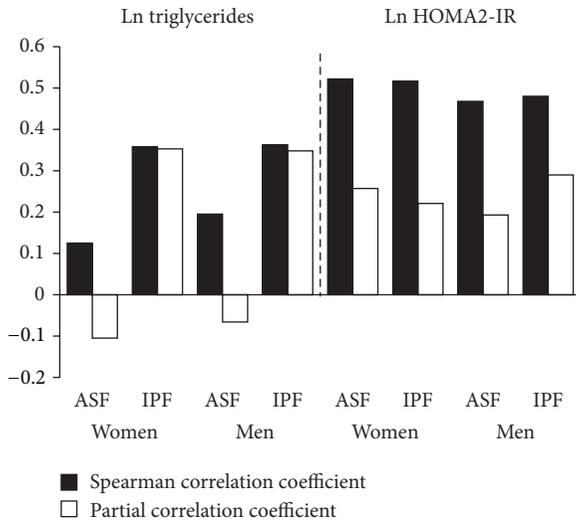


FIGURE 6: Black bars show Spearman correlation coefficients (r) for regression analyses of ln triglyceride and ln HOMA2-IR versus abdominal subcutaneous fat (ASF) and intraperitoneal fat (IPF) in women and men. White bars show partial correlation coefficients after adjustment for the opposite fat compartment. For this analysis, all men and all women were combined. For triglycerides, ASF was modestly correlated but became negatively correlated after adjustment for IPF. IPF was more strongly correlated with triglycerides and remained equally correlated after adjustment for ASF. Both ASF and IPF were strongly correlated with ln HOMA2-IR, but after adjustment for the opposing compartment, partial correlation coefficient was diminished. All correlation coefficients were significant at a level of $P < 0.001$.

occurrence of excessive amounts of IPF with obesity. The findings of Nielsen et al. [28] suggest that with increasing obesity, a higher percentage of splanchnic flux of fatty acids is derived from IPF. In addition, for any given level of obesity, there is heterogeneity in IPF content; therefore, obese persons with the greatest IPF masses could have the highest splanchnic flux of fatty acids, worsening liver-associated risk factors.

In summary, this study shows that WC correlates with IPF but not strongly. For any given WC, IPF can vary greatly. The two factors affecting this variation are ethnicity and gender. Black men and women have lower median IPF masses than whites and Hispanics. In the high WG category, blacks have both lower IPF and greater ASF, which contributes to the overall variability between WC and IPF. Both IPF and ASF contribute to metabolic risk factors. IPF was found to correlate with both serum triglyceride levels and HOMA2-IR, whereas ASF correlated only with HOMA2-IR. The mechanisms responsible for these latter correlations require further study.

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Review Article

Does Adipose Tissue Thermogenesis Play a Role in Metabolic Health?

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The function ascribed to brown adipose tissue in humans has long been confined to thermoregulation in neonates, where this thermogenic capacity was thought lost with maturation. Recently, brown adipose tissue depots have been identified in adult humans. The significant oxidative capacity of brown adipocytes and the ability of their mitochondria to respire independently of ATP production, has led to renewed interest in the role that these adipocytes play in human energy metabolism. In our view, there is a need for robust physiological studies determining the relationship between molecular signatures of brown adipose tissue, adipose tissue mitochondrial function, and whole body energy metabolism, in order to elucidate the significance of thermogenic adipose tissue in humans. Until such information is available, the role of thermogenic adipose tissue in human metabolism and the potential that these adipocytes may prevent or treat obesity and metabolic diseases in humans will remain unknown. In this article, we summarize the recent literature pertaining to brown adipose tissue function with the aims of drawing the readers' attention to the lack of data concerning the role of brown adipocytes in human physiology, and to the potential limitations of current research strategies.

1. Introduction

As the combustion engine of respiring cells, the mitochondrion is an essential component of life. Furthermore, as these organelles are principally responsible for the oxidative disposal of glucose and fatty acids, the role of mitochondrial function in the etiology of diseases where substrate metabolism is perturbed has been the focus of a considerable research effort for a number of decades. A notable example of this is the role of mitochondrial capacity in the pathogenesis of skeletal muscle insulin resistance. The reason for such interest in the role of altered skeletal muscle bioenergetics in the development of insulin resistance are several fold; skeletal muscles significant contribution to body mass, the relative abundance of mitochondria within skeletal muscle and their

plasticity (particularly in response to muscular contraction), skeletal muscles central role in whole body glucose and fatty acid disposal, and the fact that skeletal muscle can be sampled from humans relatively easily are all contributing factors. With regard to skeletal muscle, whether insulin resistance is indeed the chicken, with mitochondrial dysfunction being the preceding egg, or whether the opposite is the case remains unclear. However, what does seem clear is that measures of skeletal muscle oxidative capacity and mitochondrial content correlate well with skeletal muscle insulin sensitivity [1, 2], making them attractive outcome measures for researchers interested in insulin sensitivity.

In contrast to skeletal muscle, adipose tissue has long been considered a relatively quiescent tissue in humans, particularly with regards to its oxidative metabolism, where it

was generally thought to function as a storage depot for excess calories. While several depots of adipose tissue are present in humans (including but not limited to subcutaneous, visceral, epicardial, and brown), the two major stores, subcutaneous and visceral, both have the capacity to significantly expand in obese subjects, where it has been proposed that these two adipose tissue depots contribute differently to metabolic risk factors in humans [3]. However, as adipose tissue has far fewer mitochondria than other tissues such as liver and muscle, the role of adipose tissue mitochondrial function (if any) in human physiology has been paid very little attention to date. The principal reason for this is that, until recently, the prevailing dogma was that brown adipose tissue was only found in small animals and in human neonates, where it played a role in nonshivering thermogenesis. However, as brown adipose tissue has now been identified in adult humans [4–7], and perhaps just as intriguingly, the identification of UCP1 positive adipocytes within white adipose tissue [8], has led to renewed interest in the scientific community as to whether metabolically active (thermogenic) adipose tissue depots play a significant role in human physiology.

2. Adipose Tissue Mitochondrial Function

Interest in adipose tissue mitochondria pertains not to their role in ATP production, but rather their ability to respire independently of ATP production, that is, thermogenesis. While this process may be considered detrimental, particularly in organs with high ATP turnover rates like the heart, it allows mitochondria to produce heat as opposed to chemical energy, a significant process in mammalian thermoregulation. Unlike other tissues, brown adipocytes contain uncoupling protein 1 (UCP1), which, despite having homologues (UCP2–5) in other tissues, is the only UCP thought to have the ability to significantly uncouple the respiratory chain when activated *in vivo*. In this instance, UCP1 essentially short-circuits the electron transport chain, meaning that the intramembrane proton gradient of the mitochondrion is dissipated independently of ATP synthase. Indeed, the chemiosmotic theory of oxidative phosphorylation put forward by Mitchell [9] described how the excursion of protons from the matrix of the mitochondrion to its intramembrane space results in the formation of a proton gradient, which dissipates when protons re-enter the mitochondrial matrix via complex V of the respiratory chain, ATP synthase. ATP synthase harnesses this potential energy and uses it to phosphorylate ADP, thus producing ATP. Interestingly though, as per the laws of thermodynamics, when the electron transport chain is uncoupled, this chemical energy is not lost, rather it is dissipated as heat. As such, UCP1 was classically described as thermogenin, due to its ability to generate heat from mitochondrial respiration [10]. While the physiological role ascribed to the uncoupling of mitochondrial respiration from ATP synthesis is thermoregulation, renewed interest in the ability of UCP1 positive adipocytes to produce heat and thus increase energy expenditure relates to the potential that activation of this tissue may alter energy expenditure and body mass in humans.

3. Can UCP1 Positive Adipocytes Alter Metabolism in Humans?

The relatively recent identification of brown adipose tissue depots in humans has led to renewed interest in the scientific community as to whether metabolically active (thermogenic) adipose tissue plays a role in human body weight regulation and metabolism [11]. In our view, the two most apparent questions that remain unanswered are as follows. (1) What are the physiological roles of brown and brown-like adipocytes and (2) does the activation of brown fat *in vivo* in humans alter metabolism (basal metabolic rate, glucose disposal, and lipid profile)?

Experimentally, an abundance of data derived from murine models has evidenced that brown adipose tissue activation can alter energy metabolism *in vivo* [12, 13]. However, a broader caveat of the use of rodents, and particularly mice in metabolic research, relates to mammalian thermoregulation. In general, animal rooms in research facilities are kept at temperatures that are ambient to human beings (close to ~20°C). However, as discussed in detail by Cannon and Nedergaard [14], a thermoneutral temperature for a rodent (particularly young mice) is around 30°C. This poses a problem as it suggests that the majority of rodents used in research may be cold and thus have chronically activated brown fat. As such, this consideration makes the translation of these animal studies to human metabolism questionable.

Brown adipose tissue, with its high mitochondrial density and oxidative capacity, is more akin to skeletal muscle than white adipose tissue [15]. While skeletal muscles oxidative capacity, that is, its ability to respire and produce ATP, is considerable, this potential is only realized during near maximal muscular contraction, when ATP turnover rates increase considerably. This begs the question of whether a tissue with a similar metabolic potential, such as brown adipose tissue, can be periodically or even chronically activated in humans to an extent that significantly alters energy expenditure. Given brown adipose tissues role in thermogenesis, investigators typically expose rodents to prolonged cold exposure to demonstrate activation of brown adipose tissue [10], an approach which also works in humans [7]. However, in removing the physiological stimulus (cold exposure), one would assume then that brown adipose tissue would return to a more quiescent state. Indeed, returning cold exposed rodents to thermoneutral conditions attenuates brown adipose tissue activation, suggesting that a chronic physiological stimulus is obligatory for UCP1 activation. Moreover, it appears that UCP1 is not spontaneously leaky, being inhibited *in vivo* by purine nucleotides [16], suggesting that having brown adipose tissue alone may not alter energy expenditure.

While recent research has indeed confirmed the presence of brown adipocytes in humans, the relationship between these UCP1 positive adipocytes and energy expenditure, particularly in relation to clinical outcomes such as obesity and insulin sensitivity, remains to be seen. A limitation of the current literature is the reliance on a UCP1 signal to infer thermogenesis *in vivo* [17]. While the presence of UCP1 mRNA in adipocytes does indeed suggest a greater oxidative

capacity and, importantly, a greater capacity for uncoupled respiration, measurement of UCP1 mRNA expression alone says little about the activation of the mitochondrion or UCP1 *in vivo*. Subsequently, concurrent measures of energy metabolism and adipose tissue bioenergetics will likely play an important role in elucidating the significance of UCP1 positive fat in human physiology. With that said, careful consideration into the determination of mitochondrial function in adipocytes is needed in order to ensure that the best representation of function *in vivo* is maintained in *ex vivo/in vitro* analyses. For example, with regards to skeletal muscle mitochondrial function in insulin resistance, varied outcome measures used as markers of mitochondrial function and content employed by different laboratories have muddied the waters with regards to forming a scientific consensus [18]. Moreover, it would seem that determining mitochondrial function *in vitro* in isolated organelles versus organelles which remain *in situ* also may influence experimental results [19–21]. This may be of particular relevance to UCP1 positive adipocytes given that the UCP1 of isolated mitochondria may not be under the same level of inhibition *in vitro* as would be the case *in vivo* [22].

While data concerning bioenergetics in adipose tissue are scarce, subcutaneous adipose tissue mitochondrial respiration was first determined by Hallgren and colleagues [23]. These investigators suggested that mitochondrial respiration in adipose tissue contributed to ~4% of calculated 24 hr resting energy expenditure. In addition, they also reported that obesity and advancing age were associated with a decline in adipose respiration per gram of tissue [23]. More recently, Kraunsøe and co-workers [24] demonstrated that visceral adipose tissue contained significantly more mitochondria than subcutaneous adipose tissue from obese humans. Moreover, visceral adipose tissue mitochondria were more sensitive to substrates than their counterparts in subcutaneous adipose tissue [24]. Interestingly, the above findings suggest that adipose tissue bioenergetics are influenced by age and obesity, and that mitochondrial density and intrinsic function differs in different adipose tissue depots. While the clinical significance of these findings remain unresolved, evidence from severely burned patients suggests that brown adipose tissue may play a role in the hypermetabolic response associated with this type of trauma. For example, the marked increase in energy expenditure following thermal injury can only partly be accounted for by ATP consuming reactions, where uncoupled mitochondrial respiration has been suggested to contribute to hypermetabolism in severely burned individuals [25]. Indeed, in rodents at least, UCP1 mRNA is induced in both brown and white adipocytes following burn injury [26, 27].

4. Is the Future “Brite” for Brown Adipose Tissue?

The recent finding that humans contain brown adipose tissue depots and that thermogenic adipocytes may also reside in white adipose tissue depots has led to renewed interest in the role that UCP1 positive adipocytes play in human

metabolism [28]. The supposition that these adipocytes, which are more comparable to skeletal muscle with regards to oxidative capacity, may significantly alter energy expenditure in humans via uncoupled mitochondrial respiration is a reasonable one. For example, assuming that respiration in adipose tissue accounts for ~4% of resting metabolic rate [23], or approximately 100 kcal per day in an adult requiring 2500 kcal a day to maintain body mass, then a significant increase in the adipose tissue respiration (in the form of uncoupled respiration) over several months or even years could theoretically have a meaningful impact on body mass, adiposity, and thus metabolic health. Given the current proportion of children and adults affected by the metabolic complications associated with obesity, particularly in the developed world, the therapeutic potential of harnessing the thermogenic properties of UCP1 positive adipocytes is of great interest to both biomedical researchers and the pharmaceutical industry. However, for this potential to be realized, immediate questions as to how UCP1 positive adipocytes can be induced and/or activated *in vivo* in free living humans needs to be addressed. Detailed studies of both whole body metabolism and adipose tissue mitochondrial function in animals maintained in thermoneutral conditions, as well as in healthy humans and patient cohorts, will be central to this process.

Conflict of Interests

The authors have no conflict of interests to declare.

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Review Article

Adipose Tissue Dysfunction in Nascent Metabolic Syndrome

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The metabolic syndrome (MetS) confers an increased risk for both type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Moreover, studies on adipose tissue biology in nascent MetS uncomplicated by T2DM and/or CVD are scanty. Recently, we demonstrated that adipose tissue dysregulation and aberrant adipokine secretion contribute towards the syndrome's low-grade chronic proinflammatory state and insulin resistance. Specifically, we have made the novel observation that subcutaneous adipose tissue (SAT) in subjects with nascent MetS has increased macrophage recruitment with cardinal crown-like structures. We have also shown that subjects with nascent MetS have increased the levels of SAT-secreted adipokines (IL-1, IL-6, IL-8, leptin, RBP-4, CRP, SAA, PAI-1, MCP-1, and chemerin) and plasma adipokines (IL-1, IL-6, leptin, RBP-4, CRP, SAA, and chemerin), as well as decreased levels of plasma adiponectin and both plasma and SAT omentin-1. The majority of these abnormalities persisted following correction for increased adiposity. Our data, as well as data from other investigators, thus, highlight the importance of subcutaneous adipose tissue dysfunction in subjects with MetS and its contribution to the proinflammatory state and insulin resistance. This adipokine profile may contribute to increased insulin resistance and low-grade inflammation, promoting the increased risk of T2DM and CVD.

1. Introduction

The metabolic syndrome (MetS) comprises a cluster of cardiometabolic risk markers with insulin resistance and adiposity as central features [1–4]. Five diagnostic criteria for MetS have been identified (central obesity, dyslipidemia (high triglycerides (TGs) and/or low high-density lipoprotein cholesterol (HDL-C)), hypertension, and impaired fasting glucose) by the Adult Treatment Panel III (ATPIII) criteria of the National Cholesterol and Education Program (NCEP), and the presence of three of these features is considered sufficient to diagnose the syndrome [2, 4, 5]. Using this definition, the National Health and Nutrition Examination Survey (NHANES) data show that currently ~35% of all US adults have MetS [6] and that >40% of adults over the age of 50 have the syndrome [7]. It is important to emphasize that the diagnosis of MetS has been harmonized using the NCEP ATPIII criteria with the exception of different cut-points for waist

circumference for different races [8]. Furthermore, MetS confers an increased risk for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [7, 9–12], both of which are additional risk factors for increased morbidity and mortality.

Numerous investigators have shown increased circulating biomarkers of inflammation in MetS, thus providing support for the syndrome's proinflammatory state [2, 4, 13]. Furthermore adipokine biology has been extensively detailed in recent reviews, and hence it will not be the focus of this paper [14–16]. However, there are scant data on adipose tissue biology in individuals with nascent MetS (a term coined by us to denote subjects with MetS but without the confounding presence of diabetes and/or cardiovascular diseases) [17]. The relationship between inflammation and MetS is supported by several studies [2, 4, 18, 19], as is the relationship between increased visceral fat mass and MetS [20–22]. However, there is a paucity of data on subcutaneous adipose tissue (SAT) biology in the pathogenesis of MetS [23].

The subcutaneous fat—which comprises ~80% of adipose tissue and is the major source of fatty acids for the liver—is readily accessible to study and has been shown to be metabolically correlated to indices of insulin resistance as well as to visceral adipose tissue (VAT) [24–27]. In addition to intra-abdominal fat, investigators have shown that the amount of SAT in subjects with MetS positively correlates with increasing MetS factor scores and negatively correlates with circulating adiponectin levels [28]. Other investigators have also reported that SAT is significantly associated with MetS and increases with the increasing number of MetS features, independent of age and sex [29]. Furthermore, inflammatory cells and processes, such as macrophage infiltration, appear to be important in adipose tissue inflammation. Specifically, investigators have examined abdominal SAT from obese subjects and reported that an inflamed adipose phenotype characterized by tissue macrophage accumulation in crown-like structures (CLSs) is associated with systemic hyperinsulinemia and insulin resistance and impaired endothelium-dependent flow-mediated vasodilation [30]. Macrophage retention in fat was also linked to upregulated tissue CD68 and tumor necrosis factor- α (TNF- α) mRNA expressions in addition to increased plasma high-sensitivity C-reactive protein (hsCRP) concentrations.

Although it appears that chronic low-grade inflammation could be a central feature to explain the increased risks of CVD and diabetes in MetS, the precise mechanisms remain to be elucidated. As such, our laboratory has focused on the potential role of SAT dysregulation in the syndrome's pathogenesis. Specifically, given the paucity of data examining SAT biology and plasma adipokines in subjects with nascent MetS, we have investigated the role of SAT in MetS and its contribution to the syndrome's systemic low-grade inflammatory process.

2. Subcutaneous Adipose Tissue Dysregulation in Nascent MetS

To determine whether SAT biology in subjects with nascent MetS is dysregulated and contributes to the syndrome's systemic low-grade inflammatory process, we studied 65 age- and sex-matched adults [31]. Subjects were classified as having MetS or not using the NCEP ATP III criteria [5]; those classified as MetS had at least three risk factors to sustain the diagnosis, including central obesity, hypertension, dyslipidemia (low HDL-C and high TGs), impaired fasting glucose, and/or hypertension or on antihypertensive medications. The control subjects needed to have ≤ 2 features of MetS and not be on blood pressure (BP) medications. Other exclusion criteria for controls were a fasting plasma glucose concentration >100 mg/dL and a fasting TG concentration >200 mg/dL. For both groups, other exclusion criteria were a previous diagnosis of diabetes, clinical atherosclerosis (coronary artery disease (CAD), peripheral vascular disease, CVD, etc.), a TG concentration >400 mg/dL, a hsCRP concentration >10 mg/L, pregnancy, an increased complete blood count (CBC), alcohol consumption >1 oz/day, consumption of n-3 polyunsaturated fatty acids, smoking, hypo- or hyperthyroidism, malabsorption, active wounds, recent surgery,

inflammatory or malignant disease, anticoagulant therapy, steroid therapy, the current use of anti-inflammatory drugs, statins and/or other hypolipidemic agents, hypoglycemic agents, angiotensin receptor blockers, oral contraceptives, and antioxidant supplements (in the prior 6 months), postmenopausal women on estrogen replacement therapy, and chronic high intensity exercisers (exercise >100 min/week).

Fasting plasma samples were collected from all volunteers after informed consent, and SAT biopsies were obtained from the gluteal area. Biomarkers that were examined included adiponectin, CRP, serum amyloid A (SAA), leptin, plasminogen activator inhibitor-1 (PAI-1), retinol-binding protein-4 (RBP-4), chemokines (monocyte chemoattractant protein-1 (MCP-1), and interleukin (IL)-8), as well as cytokines (IL-1, TNF- α , IL-8, and IL-6). In addition, SAT samples were stained for CD68 (a macrophage marker) and T-cells (CD3 and CD5) to assess macrophage/T-cell infiltration into the adipose tissue, and the numbers of CLS per high power field (HPF) were counted. There were no significant differences in the ages of the participants and the male:female ratio between the controls and subjects with MetS. Since the percent of African Americans in our cohort was only 9 percent [32], we were unable to undertake any realistic subgroup analyses with respect to race.

Not surprisingly, the waist circumference (WC), body mass index (BMI), BP, fasting glucose concentrations, non-HDL-cholesterol concentrations, TG concentrations, and the homeostasis model assessment (HOMA) for insulin resistance were higher in the MetS subjects than in controls, whereas HDL-C concentrations were lower. Furthermore, the hsCRP, IL-6, IL-1 β , leptin, SAA, and RBP-4 concentrations were significantly higher in the MetS subjects than in controls, whereas the adiponectin concentrations were lower.

Table 1 shows the concentrations of several biomarkers released from incubated SAT specimens from the controls and subjects with MetS. Expressed per gram of fat, the levels of secreted leptin, RBP-4, CRP, SAA, PAI-1, and MCP-1 were significantly higher in subjects with MetS than controls. Moreover, the SAT release of IL-1 β , IL-6, IL-8, and MCP-1, as expressed per mg of protein, was higher in SAT from subjects with MetS than controls.

No lymphocyte populations were observed in any of the SAT specimens from the controls and subjects with MetS using CD3 and CD5 staining. However, there were significantly increased numbers of macrophages infiltrating the SAT of MetS subjects compared to controls as demonstrated by positive CD68 staining. Furthermore, there were significantly increased numbers (~3-fold) of CLS in the SAT specimens from MetS subjects than those from controls (controls: 5 CLS/10 hpf; MetS: 14 CLS/10 hpf; $P < 0.001$). Interestingly, the CLS did not correlate with any proinflammatory mediators, suggesting that they are not classical M1 macrophages [31]. The elucidation of the SAT macrophage phenotype in subjects with MetS is critical to understanding its role in the syndrome's pathogenesis.

Since the patients with MetS in our study cohort had significantly greater WCs than the controls, all the analytes were also evaluated with WC as a covariate. Importantly, in the adjusted analyses, all the reported differences between

TABLE 1: Subcutaneous adipose tissue biomarker levels.

	Controls	MetS
Adiponectin (ng/g)	4.2 (1.3, 5.6)	3.7 (1.2, 4.6)
Leptin (ng/g)	3.0 (2.1, 6.2)	7.3 (3.8, 18.6)*
RBP-4 (ng/g)	11.1 (6.4, 18.4)	29.1 (16.2, 33.7)**
CRP (ng/g)	2.5 (2.3, 7.9)	5.4 (3.4, 19.1)*
SAA (ng/g)	14.8 (5.1, 34.2)	25.3 (14.5, 55.7)*
PAI-1 (ng/g)	3.2 (2.2, 6.5)	5.6 (3.1, 9.9)**
MCP-1 (ng/g)	6.7 (4.3, 9.1)	22.1 (11.8, 33.5)**
IL-1 β (ng/mg protein)	31.1 (21.2, 45.1)	39.7 (24.8, 61.5)*
TNF (ng/mg protein)	3.7 (1.9, 4.6)	3.8 (2.9, 5.3)
IL-6 (ng/mg protein)	16.5 (10.6, 24.5)	18.7 (12.7, 33.2)*
IL-8 (ng/mg protein)	10.9 (5.1, 14.2)	17.4 (14.5, 27.3)**

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Data are expressed as median (25th percentile and 75th percentile).

* $P < 0.05$, ** $P < 0.001$ compared to controls.

the MetS and control groups persisted except for RBP-4 (Table 1). Furthermore, a subgroup analysis revealed higher levels of only leptin and RBP-4 in those subjects with a WC over the median, 42 in. versus under 42 in. (for WC <42 in., leptin was 71 (49–83) ng/ml versus 82 (55–89) ng/ml for WC >42 in.; for WC <42 in., RBP-4 was 25 (11–31) g/mg protein versus 33 (17–37) ng/mg protein for WC >42 in.; $P < 0.05$ compared with WC <42 in.).

Because both insulin resistance and low-grade inflammation are typical features of MetS, we used HOMA and hsCRP as the representative biomarkers for these conditions to evaluate relevant correlations as well. HsCRP correlated positively with HOMA ($r = 0.39$, $P = 0.03$) and adipose tissue MCP-1 ($r = 0.46$, $P = 0.03$) and negatively with adipose tissue adiponectin ($r = -0.44$, $P = 0.01$). There were significant correlations between circulating CRP and SAT CRP ($r = 0.49$, $P = 0.01$), IL-1 β ($r = 0.56$, $P = 0.002$), and IL-6 ($r = 0.76$, $P = 0.001$) as well. Furthermore, HOMA correlated positively with PAI-1 ($r = 0.56$, $P = 0.02$), RBP-4 ($r = 0.49$, $P = 0.03$), and SAT MCP-1 ($r = 0.039$, $P = 0.04$).

Given the sparse data on adipose tissue biology in nascent MetS, we recently reported our findings evaluating four additional novel adipokines in this cohort, including chemerin, omentin-1, resistin, and visfatin in both plasma and SAT [17]. Importantly, as noted above, none of the subjects studied had diabetes or any chronic inflammatory diseases nor were any on anti-inflammatory, hypolipidemic, or hypoglycemic drugs. Furthermore, all the subjects had CRP levels <10 mg/L, normal CBCs, fasting glucose concentrations between 100 and 125 mg/dL, and a hemoglobin A1c (HbA1c) <6.5%. Not surprisingly, subjects with MetS were more insulin resistant and had higher levels of hsCRP. It is important to emphasize that in this paper 20 percent of the patients with MetS were not obese.

The circulating levels of chemerin were significantly increased in the subjects with MetS compared to controls, and this significance persisted ($P = 0.0005$) following adjustment for age and BMI or WC (Table 2). There were also

significantly lower levels of plasma omentin-1 in subjects with MetS compared to controls ($P = 0.004$); importantly, the significance persisted when the data were adjusted for BMI or WC (Table 2) ($P = 0.03$). Furthermore, there were higher levels of circulating resistin in subjects with MetS compared to controls; however, these differences did not persist following adjustment for BMI or WC. Plasma visfatin levels were not significantly different between the two groups.

Importantly, there was also a significantly higher release of chemerin from SAT in subjects with MetS which persisted following adjustment for BMI or WC and age (Table 2). In addition, there was a significantly lower secretion of omentin from SAT in subjects with MetS which persisted following adjustment for both age and BMI or WC. However, the secretion of both resistin and visfatin from SAT was not significantly different between the MetS and control groups. Plasma chemerin concentrations correlated significantly ($P < 0.05$) with SAT chemerin ($r = 0.44$), hsCRP ($r = 0.28$), HOMA ($r = 0.42$), TG ($r = 0.41$), systolic BP ($r = 0.28$), omentin ($r = -0.42$), and HDL-C ($r = -0.37$). Moreover, both circulating and SAT omentin-1 levels correlated significantly with each other ($r = 0.44$, $P < 0.05$). Plasma omentin-1 concentrations also correlated significantly with glucose ($r = -0.38$), TG ($r = -0.48$), and HDL-C ($r = 0.52$) levels.

3. Discussion

We have shown that SAT biomarkers and architecture differ markedly in subjects with MetS compared to matched controls [31]. Specifically, we have documented that patients with nascent MetS (without the confounding conditions of diabetes and/or CVD) have increased levels of adipokines and decreased levels of adiponectin that are related to insulin resistance and inflammation. Furthermore, we have shown that this dysregulation of adipokines is not accounted for simply by increased adiposity, suggesting that other aspects of MetS contribute to both the proinflammatory and insulin resistant states of the syndrome. Also MetS should be classified as a high-risk obesity state based on our findings. Furthermore, at least 20 percent of our cohort were not obese, underscoring the high risk of metabolic syndrome in both obese and nonobese individuals.

Furthermore, we have documented a significant increase in macrophages in SAT and abundant CLS which appear to surround a hypoxic environment triggered by adipocyte death in subjects with MetS [33–35]. For unclear reasons, there were no significant correlations between CLS and biomarkers of inflammation in our studies; however, one can speculate that the CLS could be predominantly of the M2 macrophage phenotype participating in tissue remodeling. Also, we have shown that the chemokine MCP-1 is increased in SAT from MetS subjects, and, since this chemokine facilitates the homing of macrophages to such tissue depots, our data suggest that the SAT may indeed be a key player in MetS and its associated comorbidities. Moreover, because SAT is easy to access and SAT biopsies can be performed in large-scale clinical studies, and since the expression of inflammatory genes in SAT compares well with VAT [25, 26],

TABLE 2: Novel adipokine concentrations.

Variable	Controls (<i>n</i> = 30)	MetS (<i>n</i> = 45)	<i>P</i> value MetS versus control
hsCRP (mg/L)	1.3 (0.5, 4.0)	3.1 (1.6, 5.4)	0.006
HOMA-IR	1.1 (1.0, 2.8)	2.8 (1.9, 5.1)	0.0001
Plasma chemerin (ng/mL)	271 ± 53 <i>n</i> = 20	366 ± 64 <i>n</i> = 37	<0.0001 *(0.0005)
SAT chemerin (ng/mg protein)	3.05 ± 0.94 <i>n</i> = 30	3.94 ± 0.74 <i>n</i> = 45	0.001
Plasma omentin (ng/mL)	27 ± 14 <i>n</i> = 16	16 ± 5 <i>n</i> = 16	0.004 *(0.03)
SAT omentin (ng/mg protein)	0.31 ± 0.09 <i>n</i> = 30	0.22 ± 0.10 <i>n</i> = 45	0.01
Plasma resistin (ng/mL)	1.8 (1.5, 2.5) <i>n</i> = 21	2.4 (1.7, 3.1) <i>n</i> = 31	0.04 *(0.07)
SAT resistin (ng/mg protein)	0.16 ± 0.06 <i>n</i> = 30	0.17 ± 0.05 <i>n</i> = 45	NS
Plasma visfatin (ng/mL)	0.57 (0.38, 0.71) <i>n</i> = 22	0.59 (0.31, 0.96) <i>n</i> = 36	0.14 *(0.13)
SAT visfatin (ng/mg protein)	0.17 ± 0.09 <i>n</i> = 30	0.21 ± 0.1 <i>n</i> = 45	NS

* (*P* value adjusted for age and BMI).

Results are presented as mean ± standard deviation or median (25th percentile and 75th percentile).

the evaluation of SAT in subjects with and without MetS may provide novel insights into the syndrome's pathogenesis and serious sequelae.

Our data are also consistent with some but not all studies that have reported mRNA/gene expression profiles in SAT in subjects with MetS [36, 37]. Specifically, Gormez et al. reported increased mRNA levels of TNF- α and leptin but not adiponectin in SAT from subjects with MetS versus controls [36], whereas our data showed only increased amounts of SAT-secreted and circulating leptin concentrations. However, Gormez et al. studied MetS patients with CAD with 88% of the subjects having concomitant diabetes and dyslipidemia. Thus, one cannot appropriately ascribe their findings to MetS alone, and the additional confounding effects of medical conditions and medications cannot be excluded. Furthermore, Sacks et al. [37] reported no changes with respect to IL-1 β gene expression levels in SAT from 15 subjects with MetS with CAD versus controls, whereas our data (using a much larger sample size) showed an increased amount of IL-1 β released from SAT and in plasma. However, unlike in our studies, Sacks et al. [37] focused on a few selective biomarkers/biomarkers with the confounding of CAD. The reported differences could also be the result of posttranscriptional processes, and thus highlight the problem with inferring that gene expression directly correlates with protein expression, the focus of our studies since it denotes function.

The novelty of our study is that we evaluated subjects with nascent MetS (without the confounding of diabetes and/or CVD) and assayed both plasma and SAT-secreted

levels of adipokines. It is imperative to study MetS at this nascent stage in order to obtain a better understanding of the pathophysiology of this common disorder. Thus, our data suggest that these circulating adipokines function as biomediators that contribute to the increased risk for both diabetes and CVD in MetS subjects [38–40].

It is also important to note that CRP levels are significantly increased in the SAT of MetS subjects compared to controls. Although CRP appears to be predominantly produced in the liver, previous investigators have shown that there is increased CRP gene expression from adipose tissue [41] and vascular endothelium [42], a component of the stromal vascular fraction. These data thus support the notion that MetS is a proinflammatory state and that the adipose tissue of MetS contributes to the increased inflammation of these subjects.

The essential role of SAT in metabolic homeostasis has best been described in lipodystrophic syndromes, where its absence leads to ectopic fat accumulation in the liver and skeletal muscle with concomitant insulin resistance [43]. Although a deficiency in SAT has been previously associated with MetS, our data demonstrate that the SAT may also be a key player in the pathogenesis of MetS, consistent with findings from the Framingham Heart Study showing that larger volumes of SAT were associated with more cardiometabolic risk factors [22].

Another important observation from our studies is that MCP-1 appears to correlate well with insulin resistance and inflammation. Previous studies in rodents have shown that knockout of the MCP-1 receptor results in decreased hepatic

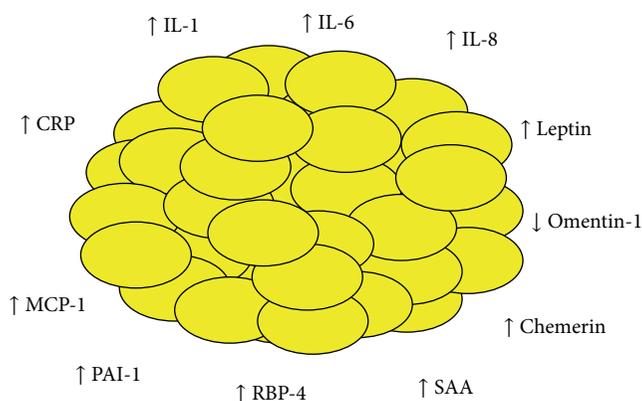


FIGURE 1: Schema depicting adipokine dysfunction in metabolic syndrome. Abbreviations: MCP-1, monocyte chemoattractant protein-1; RBP-4, retinol-binding protein-4; SAA, serum amyloid A; CRP, C-reactive protein; IL, interleukin; PAI-1, plasminogen activator inhibitor-1.

inflammation and steatosis [44] and decreased adipose tissue macrophages [45], underscoring the importance of SAT MCP-1 in MetS subjects. The exact mechanisms by which MCP-1 contributes to both insulin resistance and increased inflammation in MetS need to be elucidated in future studies.

Moreover, we have made the novel observation that the SAT in subjects with nascent MetS (uncomplicated by the comorbidities of diabetes and/or CVD) has increased macrophage recruitment with cardinal CLS in greater abundance. Furthermore, these cells in SAT conspire to produce increased levels of biomarkers that correlate with both insulin resistance and low-grade inflammation, potentially presaging the subsequent increased risk for diabetes and CVD.

In addition, we have recently demonstrated abnormal circulating and SAT-secreted chemerin and omentin-1 levels in subjects with nascent MetS [17]. Chemerin is a novel adipokine that is produced by both adipose tissue and liver; moreover, it is a chemoattractant for immune cells such as macrophages and promotes adipocyte differentiation [46]. Chemerin levels have also been shown to be higher in obesity, some features of MetS, diabetes, and nonalcoholic fatty liver disease [46–48], and it appears to induce insulin resistance in skeletal muscle, the major site of peripheral insulin resistance [49]. In our studies, we have made the novel observation that both plasma and SAT levels of chemerin are higher in subjects with nascent MetS, suggesting that chemerin could be involved early in the pathogenesis of the syndrome. Previously, in Caucasian subjects with MetS (including some with concomitant diabetes), serum chemerin levels were reported to be significantly increased; however, they were not adjusted for adiposity [50]. However, the investigators did not find a correlation between insulin resistance, obtained by two measures (HOMA and the quantitative insulin sensitivity check index (QUICKI)) and chemerin concentrations [50]. In a subsequent study in Korean subjects [51], the authors suggested that the ratio of chemerin to adiponectin might be a good predictor of MetS but did not report on adiposity-adjusted differences between patients with MetS and controls. Also, Dong et al. reported increased chemerin levels in patients with MetS (41% on statin therapy) with and without

CAD and suggested it was an independent predictor of angiographic CAD [52]. However, they too did not correct for adiposity compared to controls, and thus we are unclear if this is a manifestation of MetS per se. Nonetheless, these studies collectively suggest a role for chemerin in the pathogenesis of MetS and its use as a biomarker to predict the syndrome needs to be urgently elucidated.

Furthermore, based on our investigations, it appears that higher plasma chemerin levels in subjects with MetS emanate largely from the adipose tissue; however, we cannot exclude the contribution of other sources of chemerin production such as the liver. But, since VAT is not a major source of chemerin [53], our studies highlight the contribution of SAT to circulating chemerin levels and its use as a potential biomarker of SAT dysregulation. Moreover, our findings demonstrate higher SAT and plasma chemerin concentrations independent of obesity in nascent MetS, and also confirm significant correlations with insulin resistance, inflammation, BP, and dyslipidemia in nascent MetS, suggesting a potential role of chemerin in MetS and its sequelae [46–49].

As opposed to chemerin, omentin is predominantly expressed and secreted by VAT [54, 55] and appears to have insulin-sensitizing actions [55]. Furthermore, its levels are lower with both obesity and diabetes [55, 56]. We have documented lower levels of omentin-1 in nascent MetS in both SAT and plasma; moreover, lower omentin-1 levels persisted following correction for obesity in both plasma and in SAT [13]. Shang et al. have reported lower serum omentin-1 levels in patients with MetS (23% on statins and 32% on angiotensin-converting enzyme inhibitors/angiotensin receptor blockers) [57]; however, they did not correct for the increased BMI and waist circumference. Thus, they were unable to conclude that this correlation is a feature of MetS per se. Our study therefore adds to the published literature by documenting lower omentin release from SAT in subjects with nascent MetS independent of obesity. Moreover, since adipose tissue is the major source of omentin, we suggest that the lower secretion of omentin from SAT in subjects with nascent MetS establishes the presence of omentin deficiency in the syndrome as well.

In our studies, omentin levels significantly correlated with glucose ($r = -0.43$), TG ($r = -0.50$), and HDL-C ($r = 0.53$) concentrations, all features of MetS, but not with CRP levels and HOMA. Furthermore, although we showed higher plasma resistin concentrations in the subjects with MetS (that corrected with adiposity) versus the controls, we did not demonstrate higher SAT resistin levels. Thus, we can conclude that this cytokine, which in humans arises mainly from activated leukocytes [58], is a marker for the higher leukocyte activity that we reported previously [59, 60]. Moreover, since visfatin levels were not different in MetS versus control subjects, we cannot confirm a role for visfatin in the etiology of MetS. A schematic representation of the induced SAT dysregulation in subjects with nascent MetS is depicted in Figure 1.

4. Conclusions

We have made the novel observations that (i) the SAT in subjects with nascent MetS has increased macrophage recruitment with CLS in greater abundance; (ii) the SAT in subjects with nascent MetS produces increased levels of biomarkers that correlate with both insulin resistance and low-grade inflammation, potentially presaging the subsequent increased risk for diabetes and CVD; and (iii) there is a dysregulation in both SAT-derived chemerin and omentin-1 in subjects with nascent MetS, suggesting a possible role of these adipokines in both diabetes and CVD. Future investigations should study different fat depots to determine if our findings in a predominantly Caucasian population on SAT biology dysregulation are relevant to different race groups. Finally, comparing different fat depots in the same population with nascent metabolic syndrome will help decipher their role in adipokine dysregulation, insulin resistance, and inflammation in the pathobiology of this galloping epidemic.

Conflict of Interests

There are no potential conflict of interests related to this paper and the authors have nothing to disclose.

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Review Article

Role of Subcutaneous Adipose Tissue in the Pathogenesis of Insulin Resistance

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Burden of obesity has increased significantly in the United States over last few decades. Association of obesity with insulin resistance and related cardiometabolic problems is well established. Traditionally, adipose tissue in visceral fat depot has been considered a major culprit in development of insulin resistance. However, growing body of the literature has suggested that adipose tissue in subcutaneous fat depot, not only due to larger volume but also due to inherent functional characteristics, can have significant impact on development of insulin resistance. There are significant differences in functional characteristics of subcutaneous abdominal/truncal versus gluteofemoral depots. Decreased capacity for adipocyte differentiation and angiogenesis along with adipocyte hypertrophy can trigger vicious cycle of inflammation in subcutaneous adipose tissue and subsequent ectopic fat deposition. It is important to shift focus from fat content to functional heterogeneity in adipose tissue depots to better understand the relative role of subcutaneous adipose tissue in metabolic complications of obesity. Therapeutic lifestyle change continues to be the most important intervention in clinical practice at any level of increased adiposity. Future pharmaceutical interventions aimed at improving adipose tissue function in various subcutaneous depots have potential to help maintain adequate insulin sensitivity and reduce risk for development of insulin resistance complications.

Prevalence of obesity has been increasing in the US. In 1960s, prevalence of obesity was approximately 13% [1]. The most recent analyses of National Health and Nutrition Examination Survey (NHANES) reported that 33.8% of adults (age 20 years or more) and 16.8% of children and adolescents (age 2–19 years) are obese [2, 3]. Obesity is associated with increased morbidity and mortality and decreased life expectancy. Obesity is associated with increased risk for cardiovascular diseases. These include coronary heart disease, heart failure, and sudden death [4, 5]. In fact heart disease is the leading cause of death (1 in every 4 deaths) for both men and women in the USA [6]. In addition to cardiovascular diseases, obesity is associated with numerous other medical conditions including type 2 diabetes, dyslipidemia, hypertension, nonalcoholic fatty liver disease, cancers, and sleep apnea [4]. Insulin resistance is the key underlying pathophysiologic process for development of many of these comorbidities. Medical costs associated with obesity have increased and were estimated at 147 billion dollars in 2008 [6].

However, we have to consider clinical paradoxes along the spectrum of obesity, insulin resistance and metabolic complications. Metabolically healthy but obese (MHO) phenotype exhibits higher insulin sensitivity, absence of hypertension, and favorable lipid, inflammation, hormonal and liver enzyme profile. On the basis of epidemiological and clinical studies, prevalence of MHO phenotype varies from 10%–40% [7]. The extent to which this favorable metabolic profile translates into decreased risk of cardiovascular disease and mortality is unclear. Some studies have reported that MHO phenotype is not at increased risk for cardiovascular disease [8, 9]. However, Arnlov et al. [10] reported that obese men without metabolic syndrome were at increased risk for cardiovascular events and death compared to normal weight individuals without metabolic syndrome. Kuk and Ardern et al. [11] reported that obese individuals, with or without metabolic risk factors, had increased mortality compared to nonobese individuals. This has very important implication in clinical practice. Therapeutic lifestyle change including

weight loss and physical activity is still important for obesity-associated comorbidities like osteoarthritis and sleep apnea and reducing mortality from obesity itself. Second paradox is that of metabolically obese but normal weight or normal weight obesity. This phenotype is characterized by not being obese on the basis of height and weight but with hyperinsulinemia, insulin resistance, increased risk for type 2 diabetes, hypertriglyceridemia and atherosclerosis [12]. Recently, Romero-Corral et al. [1] analyzed 6171 individuals >20 years of age from NHANES III survey and NHANES III mortality study and found that subjects who had normal body mass index but had high fat content had high prevalence of cardiometabolic dysregulation, metabolic syndrome, and cardiovascular risk factors. Based on the latest US census and obesity prevalence data, the authors estimated that normal weight obesity is present in approximately 30 million Americans [1, 13]. We have previously reported that migrant Asian Indians, compared to non-Hispanic white Americans, have excessive insulin resistance relative to their degree of obesity [14]. From personal and public health standpoint, it is important to recognize cohort with normal weight obesity and introduce therapeutic lifestyle changes and aggressive risk factor modification.

Excessive insulin resistance and related metabolic abnormalities can be due to differential distribution of adipose tissue and/or adipose tissue dysfunction.

Anatomically adipose tissues can be divided into truncal region or peripheral region. Truncal adipose tissue includes subcutaneous fat in thoracic and abdominal region and also intrathoracic and intraabdominal fat depots [15]. Peripheral adipose tissue includes subcutaneous depots in upper and lower extremities. Whether accumulation of adipose tissue in a particular region contributes to increased risk of development of insulin resistance and metabolic consequences is controversial.

Vague in 1947 described two patterns of adipose tissue distribution—android (upper body) and gynoid (lower body)—and suggested that android obesity was associated with diabetes, coronary artery disease, gout, and uric acid renal stones [15]. Later on many epidemiological studies assessing regional adiposity using waist to hip circumference ratio have reported that increased waist to hip circumference ratio is associated with hyperinsulinemia, impaired glucose tolerance, type 2 diabetes, hypertriglyceridemia, hypercholesterolemia, hyperuricemia, and atherosclerotic vascular disease [15]. However, we have to keep in mind that elevated waist circumference does not always indicate increased visceral adiposity but instead can also indicate increased subcutaneous adiposity. Adipose tissue in different depots can have different physiological characteristics and can have different impact on metabolic risk. Many investigators have reported that intraabdominal (visceral) adipose tissue is a major contributor to metabolic risk [16–18] whereas some investigators have suggested that subcutaneous adipose tissue may have a protective role [19].

Visceral fat has increased metabolic activity, both lipogenesis and lipolysis, compared to other fat depots. Free fatty acids, product of lipolysis, can directly enter liver via portal circulation and lead to increased lipid

synthesis, gluconeogenesis, and insulin resistance resulting in hyperlipidemia, glucose intolerance, hypertension, and ultimately atherosclerosis [16]. Excess free fatty acids can induce peripheral insulin resistance by inhibiting skeletal muscle uptake [15]. However, if visceral fat was a major contributor to metabolic risk, visceral fat, in comparison to other fat depots, should be the major source of systemic free fatty acid flux. Only small portion of total body fat, 15%–18% in men and 7%–8% in women, is located in abdominal cavity [20]. Visceral fat contributes to only 15% of the total systemic free fatty acids whereas the majority of free fatty acids are contributed by nonsplanchnic adipose tissue [15, 21]. This raises doubt over contribution of visceral fat to peripheral insulin sensitivity.

We have examined the relationships between generalized and regional adiposity and insulin sensitivity in a group of nondiabetic men with varying degree of obesity [22]. We concluded that subcutaneous truncal fat plays a major role in obesity-related insulin resistance in comparison to intraperitoneal (visceral) or retroperitoneal fat. Subsequently, we examined similar relationship among men with noninsulin-dependent diabetes mellitus (NIDDM) [23]. We found that NIDDM men had a fat distribution pattern that favors truncal subcutaneous depot than peripheral subcutaneous or intraperitoneal fat depot. Besides, truncal subcutaneous fat had a stronger correlation with insulin sensitivity than intraperitoneal fat among NIDDM men. Along the same line, Goodpaster et al. [24] have also demonstrated stronger relationship between subcutaneous abdominal fat and insulin sensitivity. Cross-sectional analysis of data from the Amsterdam Growth and Health Longitudinal Study by Ferreira et al. [25] revealed that high subcutaneous trunk fat was associated with arterial stiffness.

Simple explanation for stronger relationship between subcutaneous adipose tissue and insulin sensitivity comes from larger volume of subcutaneous adipose tissue mass. The subcutaneous abdominal fat mass is approximately twice more than intraperitoneal fat mass and total subcutaneous truncal fat mass can be 4–5 times larger than intraperitoneal fat mass [22, 23, 26]. Similarly in women, subcutaneous abdominal fat area at L4–L5 level is approximately five times more than visceral fat area at the same level [26–28]. With assumption of equal metabolic activity in subcutaneous truncal and intraperitoneal fat, subcutaneous truncal fat should release more free fatty acids in systemic circulation and should have much larger impact on peripheral insulin sensitivity. As we mentioned earlier, major contributor of free fatty acids in systemic circulation is nonsplanchnic adipose tissue.

Inflammation in adipose tissue has been identified as a mediator of systemic insulin resistance. This has been suggested by presence of macrophage in the form of crown-like structures (CLSs) in adipose tissue. Apovian et al. [29] examined relationships between adipose macrophage infiltration and insulin resistance and vascular endothelial dysfunction in obese individuals. In comparison to noninflamed subcutaneous abdominal adipose phenotype, inflamed phenotype, characterized by presence of macrophage in crown-like structures, was associated with systemic hyperinsulinemia, insulin resistance, impaired endothelium-dependent flow-mediated vasodilatation, and elevated plasma hs-CRP levels. Other

investigators have also reported similar association between macrophage infiltration of subcutaneous adipose tissue and insulin resistance and low grade systemic inflammation [30, 31]. In the study by Lê et al. [31], influence of subcutaneous adipose tissue (SAT) inflammation on hepatic fat fraction, visceral adipose tissue, insulin sensitivity, beta cell function, and SAT gene expression was examined. SAT inflammation, independent of total adiposity, was associated with partitioning of fat towards visceral adipose tissue and the liver and altered beta cell function. In addition, several genes belonging to nuclear factor- κ B stress pathway were upregulated suggesting stimulation of inflammatory mediators. Lundgren et al. [32] examined relationship between fat cell size and insulin sensitivity. Enlarged adipocytes were found in patients with type 2 diabetes and prediabetic individuals and were an independent marker of insulin resistance in prediabetic subcutaneous adipose tissue. Presence of large adipocytes is an indicator of decreased adipogenic potential in subcutaneous tissue and can be the trigger for increased macrophage infiltration and inflammatory process activation. In fact among type 2 diabetes subjects, preadipocytes isolated from subcutaneous abdominal fat biopsies displayed decreased expression of genes involved in differentiation [33]. This is suggestive of decreased adipogenesis and decreased formation of adipocytes in subcutaneous fat depots. On the same line, Goedecke et al. [34] reported association between decreased insulin sensitivity and reduced subcutaneous adipose tissue expression of adipogenic and lipogenic genes among obese black South African women. Previously, we have reported that migrant South Asians, compared to Caucasians, have excess insulin resistance without increase in intraperitoneal fat mass. They had increased adipocyte size in the subcutaneous abdominal adipose tissue and also low grade systemic inflammation indicated by elevated plasma hs-CRP [14, 35].

Recent work on angiogenesis in adipose tissue has provided important insights into potential mechanisms of heterogeneity in the systemic metabolic impact of specific adipose tissue compartments. Gealekman et al. [36] reported that angiogenic capacity of subcutaneous abdominal adipose tissue decreased with increasing body mass index but it did not change in visceral adipose tissue. In addition, decrease in angiogenic capacity correlated with insulin resistance which suggests that impairment in subcutaneous adipose tissue angiogenesis may contribute to metabolic complications of obesity. Whether angiogenic capacity is linked to decreased adipogenic potential of subcutaneous abdominal adipose tissue remains to be established.

In contrast to abdominal subcutaneous adipose tissue, larger subcutaneous thigh fat mass has a protective effect. The Health, Aging, and Body Composition Study [37] reported that large subcutaneous thigh fat was independently associated with more favorable glucose (in men) and lipid profile (in both genders). The Australian Diabetes, Obesity, and Lifestyle Study [38] examined association between waist and hip circumferences to components of metabolic syndrome. After adjustment for age, body mass index, and waist circumference, a larger hip circumference was associated with a

lower prevalence of undiagnosed diabetes and dyslipidemia. Association with undiagnosed hypertension was weaker.

Finally, it is worth putting truncal/abdominal subcutaneous adipose tissue characteristics all together to explain their emerging role in insulin resistance [39]. Increase in caloric intake results in increase in fat accumulation in subcutaneous adipocytes. This will continue till a “tipping point is reached” and “buffering” function of subcutaneous adipose tissue does not adequately match demand for triglyceride deposition. This happens when increased adipocyte size sets up inflammatory reaction and new adipocytes recruitment and maturation is decreased. Inflammatory reaction is characterized by activation of nuclear factor- κ B pathway. The resultant effects are downregulation of cellular insulin signaling, recruitment of additional macrophages through monocyte chemoattractant protein 1, propagation of inflammation by interleukins and tumor necrosis factor alpha, and tissue matrix remodeling through matrix metalloproteinase-9 [31]. These inflammatory mediators as well as yet not well-defined metabolic pathways may limit recruitment and maturation of new adipocytes. Persisting excessive caloric intake will be associated with spillover of fatty acids and triglyceride deposition in ectopic tissues such as visceral adipose tissue and liver. At this point, variability in visceral fat mass and hepatic fat content will become the best correlate of insulin resistance. It is possible that depending on environmental and genetic factors, “tipping point” may be reached at different levels of adiposity, including in nonobese range, and it may not be reached at all even with morbid obesity. It is possible that increased fat in subcutaneous depot relative to visceral depot may not be accompanied by increase in insulin resistance since “tipping point” is not reached. This can explain discrepancy in the literature regarding differential fat distribution and insulin resistance.

Conclusion. There is enough evidence in the literature for association between obesity and insulin resistance. Many investigators have proposed that visceral adipose tissue is a major contributor to insulin resistance. Our previous studies, in concordance with those of other investigators, suggest that subcutaneous truncal adipose tissue has significant impact on development of insulin resistance. Thus, adipose tissue distribution and function in different body compartments can be heterogeneous and can differentially contribute to insulin resistance. Changing focus from visceral adipose tissue mass as a sole contributor to insulin resistance to functional heterogeneity in adipose tissue depots can help better understand relationship of adiposity and insulin resistance. Therapeutic lifestyle change, including physical activity and weight loss, continues to be the most important intervention at any level of adiposity. One can envision that better understanding of adipose tissue function in various depots will help identify much needed additional and more effective therapeutic modalities to improve adipose tissue function and maintain adequate systemic glucose and lipid metabolism to reduce risk for morbidity and mortality associated with insulin resistance.

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Review Article

The Role of the Immune System in Obesity and Insulin Resistance

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The innate immune system provides organisms with rapid and well-coordinated protection from foreign pathogens. However, under certain conditions of metabolic dysfunction, components of the innate immune system may be activated in the absence of external pathogens, leading to pathologic consequences. Indeed, there appears to be an intimate relationship between metabolic diseases and immune dysfunction; for example, macrophages are prime players in the initiation of a chronic inflammatory state in obesity which leads to insulin resistance. In response to increases in free fatty acid release from obese adipose depots, M1-polarized macrophages infiltrate adipose tissues. These M1 macrophages trigger inflammatory signaling and stress responses within cells that signal through JNK or IKK β pathways, leading to insulin resistance. If overnutrition persists, mechanisms that counteract inflammation (such as M2 macrophages and PPAR signaling) are suppressed, and the inflammation becomes chronic. Although macrophages are a principal constituent of obese adipose tissue inflammation, other components of the immune system such as lymphocytes and mast cells also contribute to the inflammatory cascade. Thus it is not merely an increased mass of adipose tissue that directly leads to attenuation of insulin action, but rather adipose tissue inflammation activated by the immune system in obese individuals that leads to insulin resistance.

1. Introduction

The obesity epidemic in the USA continues to expand at an alarming rate, with a 75% increase in prevalence since 1980 [1]. The Centers for Disease Control and Prevention (CDC) reports that more than one-third of USA adults and over one-sixth of children and adolescents are obese. The frequencies of other metabolic disorders have increased *pari passu*, including dyslipidemia, nonalcoholic steatohepatitis, and type 2 diabetes. Dysfunctional adipose tissue is central to all these conditions. Adipose tissue is increasingly recognized as a complex endocrine organ and not merely a depot for storage of fat. Adipose tissue in obese persons develops an inflammatory milieu which ultimately leads to insulin resistance. Although many components of the immune system have been found to play a role in either promoting or attenuating adipose tissue inflammation, macrophages are key players. This paper discusses the various stimuli and networks that lead to insulin resistance, with a primary focus on the role of macrophages in adipose tissue inflammation.

2. Macrophage Accumulation in Visceral Adipose Tissue in Obesity

Adipose tissue comprises not only adipocytes but also a heterogeneous constellation of adipocyte precursors, nerve terminals, blood vessels, and leukocytes collectively termed the “stromal vascular compartment” (SVC). In 2003, pioneering studies by Xu et al. [2] and Weisberg et al. [3] demonstrated that obesity is associated with significant increases in macrophage number within the SVC of visceral adipose tissue (VAT). Weisberg et al. discovered the increase in macrophage number through gene expression profiling of VAT from multiple obese mouse models and lean controls. They found that levels of roughly 1300 genes directly correlated with body mass, and of the 100 most significantly correlated genes, 30% were macrophage-related [3]. Detailed analysis of SVC via flow cytometry revealed that macrophages make up approximately 40% of SVC cells from obese rodents, compared to only 10% of SVC cells in lean litter mates [3].

To understand the role of macrophages in adipose tissue insulin resistance, it is important to distinguish visceral fat from subcutaneous fat. Visceral fat is found in association with internal organs such as omentum, mesentery, and perinephric adipose tissue [4]. Visceral adiposity is predictive of hepatic steatosis, cardiovascular disease, and type 2 diabetes, whereas an increase in the mass of subcutaneous fat appears to pose little or no risk of these conditions [4]. Visceral and subcutaneous fat also differ in immune cell composition, particularly macrophages [5–7]. Similar to mouse models, increased macrophage accumulation has been demonstrated in the adipose tissue of obese humans and those with type 2 diabetes, with significantly more macrophages residing in visceral omental fat depot compared to subcutaneous inguinal depot in these subjects [5, 6]. Additionally, while macrophage content increases in both visceral and subcutaneous fat depot following a high-fat diet (HFD) feeding, the increase is severalfold greater in VAT [4]. Overall these findings indicate that increased macrophage accumulation in VAT may be a key pathologic feature of obesity and thereby of associated conditions such as type 2 diabetes, cardiovascular disease, and fatty liver.

3. Lipolysis, Resulting in an Increase of Free Fatty Acids, Promotes Adipose Tissue Macrophage Accumulation

What is the underlying factor that promotes the accumulation of adipose tissue macrophages (ATMs)? Kosteli et al. proposed that this phenomenon may be driven by alterations in adipose metabolic function and substrate fluxes, specifically increased concentrations of free fatty acids (FFAs) [8]. They suggested that obesity causes an increase in basal lipolysis, and the resultant increase in local extracellular free fatty acid concentrations could provide a chemotactic stimulus for entry and accumulation of macrophages. Basal lipolysis is chronically elevated in adipose tissue of obese compared with lean persons and in intra-abdominal compared with subcutaneous adipose tissue [8]. Consistent with this hypothesis, visceral adipose tissue depots contain more ATMs than abdominal subcutaneous depots [8]. Many details of this process remain to be clarified. Basal lipolysis could release a number of potential signaling molecules that could play a role in macrophage chemotaxis, for example, arachidonic acid products [8]. An additional intriguing feature is that lipolysis also increases during weight loss, and ATMs recruited in response to weight loss have a more anti-inflammatory phenotype (see below) than those in the adipose tissue of stably obese animals [8]. These differences suggest that additional factors are required for the entering macrophages to take on a proinflammatory phenotype.

4. Recruitment of Macrophages in Adipose Tissue

Recruitment of macrophages into adipose tissue is an early event in obesity-induced adipose depot inflammation.

However, it is only one of several early events—overnutrition also causes adipocytes to release chemokines, such as monocyte chemoattractant protein-1 (MCP-1), providing a chemotactic gradient that attracts monocytes into adipose tissue [9]; these presumably can transform into tissue resident macrophages, that is, ATMs. A variety of chemokines released from ATMs can then recruit additional monocytes/macrophages, promoting a feed-forward process [9]. For instance, MCP-1, secreted primarily by macrophages and endothelial cells, is also secreted by adipocytes [10]. The MCP-1 ligand has high affinity for C-C motif receptor 2 (CCR2) on macrophages, and signals generated by this pathway stimulate macrophage migration into inflamed or damaged tissues [10, 11]. CCR2(–/–) mice display reduced ATM content, reduced proinflammatory cytokines, and improved systemic insulin sensitivity relative to body weight-matched wild-type (WT) controls [11]. There were some confounding phenotypic changes in the CCR2(–/–) mice; however Oh et al. showed that transplanting monocytes from the CCR2(–/–) mice into WT mice or WT monocytes into MCP-1(–/–) mice also resulted in decreased ATM accumulation [12]. Similar effects were observed when obese wild-type mice were treated with a CCR2 antagonist for a short term [11]. Conversely, transgenic mice overexpressing MCP-1 in adipocytes exhibited increased ATM levels, hepatic steatosis, and insulin resistance [13].

Another critical mediator of ATM accumulation is $\alpha 4$ integrin, which permits macrophage adhesion to endothelial cells and their subsequent transmigration through the endothelial barrier. Mice carrying a loss-of-function $\alpha 4$ integrin point mutation had reduced monocyte infiltration into VWAT and were protected from obesity-induced insulin resistance [14]. Cbl-associated protein (CAP), a known regulator of glucose transport (GLUT4) in adipocytes, also promotes macrophage mobility [15]. When the CAP gene is deleted from macrophages *in vivo*, the knock-out (KO) mice show a decrease in ATM content with reduced tissue inflammatory markers and cytokine concentrations [16]. The chemokine LTB4 is a potent chemoattractant for neutrophils—it is mainly produced by leukocytes, but is also expressed by adipocytes, and it augments MCP-1 expression in human monocytes, thus contributing to ATM infiltration [17]. Recent studies by Spite et al. have shown that mice lacking the gene encoding the LTB4 receptor, BLT1, manifested decreased inflammation in adipose tissue and liver and were protected from systemic glucose and insulin intolerance compared to WT littermates [18]. In keeping with these findings, deletion of other monocyte chemokine receptors such as CCR5 also mitigates ATM accumulation in HFD-fed mice. CCR5 and its ligands were robustly upregulated in VWAT of HFD-induced and genetically induced obese mice [19]. However, CCR5(–/–) mice were protected from insulin resistance, glucose intolerance, and hepatic steatosis induced by HFD feeding [19]. Taken together, these findings indicate that multiple chemoattractants draw macrophages, monocytes, and neutrophils into adipose tissue. An intriguing, unstudied possibility is whether these multiple chemoattractant-receptor pairs recruit different populations of macrophages or immune cells into adipose tissues.

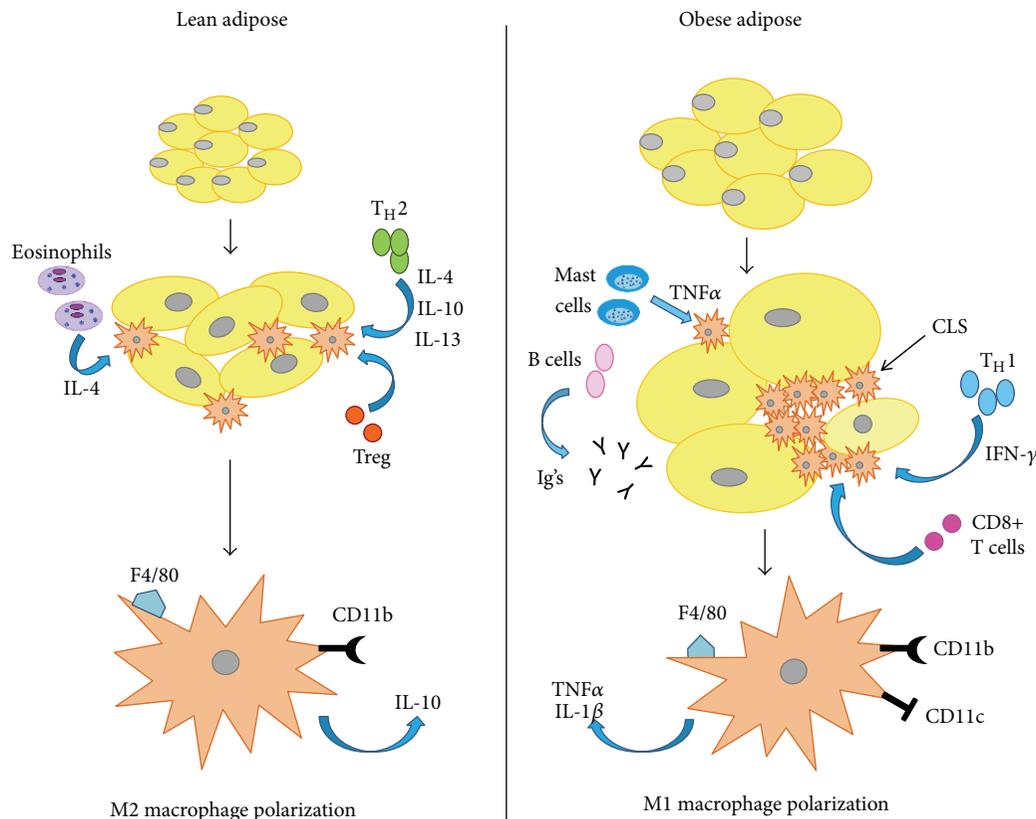


FIGURE 1: Role of the immune system in lean versus obese adipose tissue. In lean adipose tissue, T-helper type 2 (T_H2) cells produce anti-inflammatory cytokines such as interleukin (IL)-4, 10, and 13 which promote alternatively activated M2 macrophage polarization. M2 polarization is also induced by regulatory T cells (Tregs) and eosinophils via IL-4. M2 macrophages secrete other anti-inflammatory signals such as IL-10 which maintain insulin sensitivity within lean adipose tissue. Conversely, T_H1 type cytokines such as interferon (IFN)- γ stimulate M1 macrophage polarization in obese adipose tissue. Other immune cells are also increased in obese adipose tissue which contribute to insulin resistance including mast cells, B cells, and immunoglobulins (Igs). CD8(+) T cells promote ATM accumulation and proinflammatory gene expression and are also increased as well. Macrophages are not homogeneously distributed throughout obese adipose tissue but rather aggregated around dead adipocytes forming crown-like structures (CLS). M1 macrophages are proinflammatory, secreting cytokines such as TNF- α and IL-1 β . Macrophages are bone-marrow-derived myeloid cells hence both M1 and M2 macrophages express the myeloid cell surface markers F4/80 and CD11b. However, only the M1 population expresses the marker CD11c.

5. Heterogeneity of Adipose Tissue Macrophages

ATMs that reside in lean adipose tissue differ from those in obese adipose tissue. Classically activated macrophages (CAMs), termed M1, are generally stimulated by T-helper-1-type cytokines such as IFN- γ or bacterial by-products. M1 macrophages are proinflammatory, secreting cytokines such as TNF- α and IL-1 β , and have high phagocytic and bactericidal potential [20]. M1 cells are generally recruited from the circulation in a CCR2-dependent manner, so it is likely that their accumulation in adipose tissues is possibly due to increased entry from the circulation. In contrast, T-helper-2-type cytokines such as interleukins (IL)-4, 10, and 13 promote alternatively activated macrophages termed M2. M2 macrophages have antiparasitic functions, secrete anti-inflammatory cytokines such as IL-10, and function in tissue repair and remodeling [21]. M2 cells are thought to be derived from replication of ATM resident macrophages—hence it is

possible, but unproven, that their increase in adipose tissue is due to accelerated local multiplication (Figure 1).

Tissue macrophages respond to alterations in the local environment by changing their polarization status. Obesity not only promotes infiltration and migration of macrophages, but also induces a shift in macrophage balance towards the M1 phenotype [22]. In fact, obesity shifts the adipose M2 : M1 ratio from 4 : 1 in normal mice to 1.2 : 1 [23]. This shift is evident in the expression level of CD11c on ATMs. Macrophages are bone-marrow-derived myeloid cells; hence both M1 and M2 macrophages express the myeloid cell surface markers F4/80 and CD11b. However, only the M1 population expresses the marker CD11c, whereas M2 macrophages are CD11c(-) [24, 25]. M1, CD11c(+) recruited macrophages account for the majority of the increase in ATMs observed in obese adipose tissue, where >90% of recruited monocytes become CD11c(+) ATMs [24, 25]. Also, M1 correlates with insulin resistance as demonstrated by Patsouris et al. [26]. These investigators deleted CD11c(+) macrophages in mice using

a genetic system in which the primate diphtheria toxin receptor (DTR) gene is driven by the CD11c promoter; the intention was to make CD11c(+) cells expressing DTR on their surface undergo apoptotic death when the animal was exposed to diphtheria toxin. In mice fed HFD for 16 weeks, diphtheria toxin exposure ablated about half of all ATMs, while also reducing myeloid cell content in the liver and skeletal muscle. Remarkably, only 24–48 hours after diphtheria toxin administration, glucose tolerance tests completely normalized in these mice, associated with improved insulin sensitivity in both liver and skeletal muscle [26]. This study illustrated that CD11c(+) macrophage populations (M1) are responsible for insulin resistance in obese animals and demonstrated that their continued presence is required to maintain this state.

ATM M1 polarization status is not necessarily permanent; it can revert to M2 predominance under certain circumstances. For instance, switching mice from a HFD to a chow diet [27] or treating obese mice with thiazolidinediones (TZDs) [28] changed ATM polarization from M1 to M2 and subsequently improved insulin sensitivity. Although switching HFD to chow feeding reversed adipose tissue inflammatory cytokine levels, it did not change the quantity of CD11c(+) cells until 5 weeks after the diet change, indicating that the macrophage phenotype may be dynamic [27]. Furthermore, Shaul et al. demonstrated that not all CD11c(+) cells reflect the typical M1 phenotype as defined *in vitro* and in conditions of acute inflammation [29]. However, to summarize current understanding of a complex and emerging paradigm of adipose inflammation, M2 predominant ATMs are typically seen in normal, lean subjects while a transformation to the M1 state propagates the inflammatory state associated with obesity.

6. Adipocyte Death and Crown-Like Structures

Macrophage accumulation in VAT occurs in the context of continuous tissue remodeling that is pathologically accelerated in the obese state. Adipocytes increase and decrease in size in order to accommodate changes in lipid load during minor fluctuations in weight. With excessive weight gain, extreme increases in adipocyte size are accompanied by an elevated frequency of adipocyte death and macrophage accumulation [30]. The accelerated adipocyte death rate could partly be explained by hypoperfusion causing an inadequate supply of oxygen in the face of expanding adipose tissue. This phenomenon of poorly oxygenated adipose tissue was first observed in mice but has also been shown in obese humans [31]. Hypoxia activates the transcription factor hypoxia-inducible factor-1 α (HIF-1 α), which induces expression of various target genes; deletion of HIF-1 α in adipocytes partially protects mice from HFD-induced obesity and insulin resistance compared with similarly fed wild-type controls [32, 33]. Strissel et al. tracked the adipocyte death rate in obese, HFD mice by assessing VAT histology periodically for 20 weeks and found that the proportion of dead adipocytes increased from <1% of total cells to >20% during the course of the study [30]. The adipocyte death rate was associated with

parallel increases in weight, numbers of ATMs (expressing CD11c), TNF α and MCP-1 levels, and insulin resistance [30].

Macrophages are not homogeneously distributed throughout VAT but rather aggregated around dead adipocytes as shown in Figure 1. Dead adipocytes lack unilocular lipid droplets and therefore do not stain for perilipin [34]. Clusters of F4/80-staining macrophages surrounding perilipin (–) adipocytes are termed “crown-like structures” (CLS) [35]. Individual CLS contain up to 15 macrophages and the majority of ATMs are localized to CLS [35]. While it is rare to see CLS in chow-fed mice, there is greater than 10-fold increase in CLS number in HFD-fed mice [36]. In genetic and HFD models of rodent obesity, CLS are more numerous in visceral compared to subcutaneous depots, and CLS number correlates directly with insulin resistance [4, 35, 36]. The same holds true in obese humans, in whom CLS are significantly enriched in omental compared to inguinal adipose tissue [5]. Furthermore, the number of omental depot CLS correlates with local levels of inflammatory mediators, insulin resistance, and systemic vascular endothelial dysfunction, in humans [5, 37]. Taken together, CLS are pathological lesions in VAT of obese subjects, and they are highly correlated with adipose inflammation and insulin resistance.

7. Inflammatory Signaling and Stress Responses Causing Insulin Resistance

Obesity-associated insulin resistance (IR) is consistently associated with elevated levels of proinflammatory cytokines such as TNF α , IL-6, and IL-1 β , and neutralization of TNF α improves insulin sensitivity in obese rodents [38]. Both adipocytes and the M1 subset of ATMs are a major source of these cytokines. These cytokines activate inflammatory pathways that terminate in activation of Jun N-terminal kinase-1 (JNK1) and inhibitor of κ B kinase (IKK β), the products of which alter signaling downstream of the insulin receptor and cause insulin resistance [39, 40]. Activation of these kinases in obesity highlights the intertwined relationship of metabolic and immune pathways; JNK and IKK are the same kinases that are activated in the innate immune response, mediated by Toll-like receptor (TLR) signaling stimulated by lipopolysaccharide (LPS), peptidoglycan, double-stranded RNA, and other microbial products (Figure 2).

7.1. IKK β Signaling Pathway. IKK β can impact insulin signaling through at least two pathways. First, it can directly phosphorylate insulin receptor substrate protein-1 (IRS-1) on serine residues, leading to attenuation of tyrosine kinase-mediated signaling from the insulin receptor, interference of normal insulin action, and subsequent insulin resistance [39]. Secondly, IKK β leads to phosphorylation of the inhibitor of nuclear factor- κ B (I κ B). In the resting unphosphorylated state, I κ B forms a complex with nuclear factor- κ B (NF- κ B), preventing it from entering the nucleus. However, phosphorylated I κ B dissociates from NF- κ B and undergoes degradation; free NF- κ B translocates to the nucleus, binds to DNA, and activates inflammatory mediators such as TNF α and IL-6 [41]. Arkan et al. showed that mice lacking IKK β

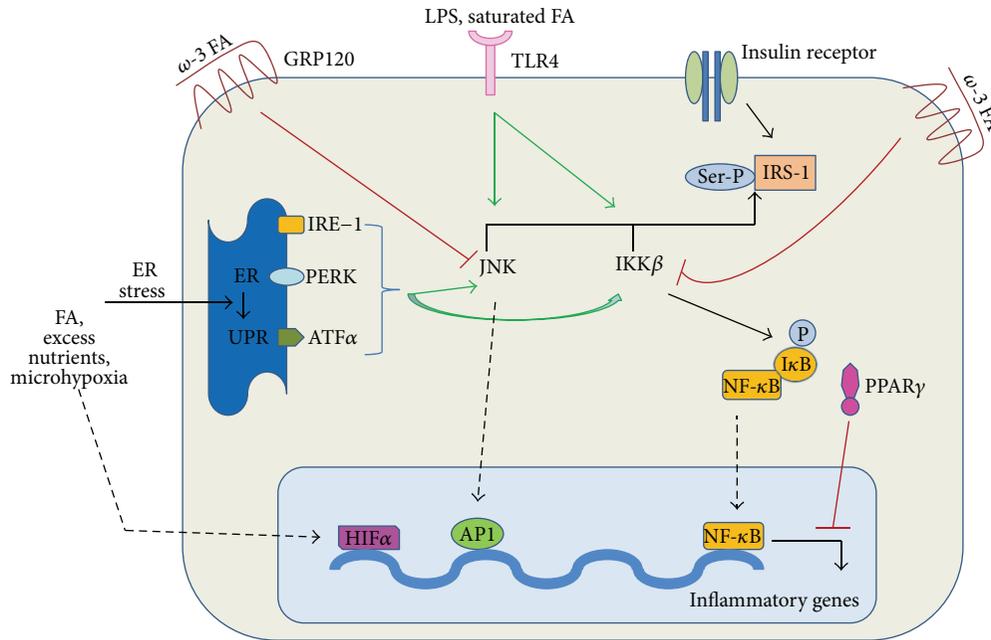


FIGURE 2: Various signaling pathways promoting or inhibiting inflammatory signaling (green arrows represent activation, and red arrows represent inhibition). Jun N-terminal kinase-1 (JNK1) and inhibitor of κB kinase (IKKβ) are inflammatory signaling pathways which promote insulin resistance. Activation of either pathway leads to serine phosphorylation of insulin receptor substrate protein-1 (IRS-1), causing attenuation of insulin action. IKKβ also phosphorylates inhibitor of nuclear factor-κB (IκB) which essentially frees nuclear factor-κB (NF-κB), allowing it to translocate to the nucleus, bind to DNA, and activate inflammatory mediators. JNK1 can also stimulate transcription of inflammatory genes in association with transcription factor activator protein 1 (AP1). Toll-like receptor 4 (TLR4) activation, which normally binds lipopolysaccharides (LPS) and saturated fatty acids (FA), results in activation of JNK and IKKβ. Endoplasmic reticulum (ER) stress, stimulated by FA, nutrient excess, and microhypoxia, leads to the unfolded protein response (UPR). UPR comprises three main pathways: inositol-requiring enzyme (IRE)-1, protein kinase-like ER kinase (PERK), and activating transcription factor (ATF)α which all lead to activation of JNK1 and IKKβ. Hypoxia also activates the transcription factor hypoxia-inducible factor-1α (HIF-1α), which induces expression of various target genes. Conversely, insulin sensitivity is promoted by activation of the omega-3 fatty acid receptor (GRP120) which inhibits JNK1 and IKKβ. PPARγ also promotes insulin sensitivity by interfering with the NF-κB and AP1 signaling pathways and subsequent expression of inflammatory genes.

in myeloid cells are insulin sensitive [42]. Moreover, when these myeloid-specific IKKβ knockout mice were placed on HFDs, they became just as obese as their wild-type counterparts but were protected from obesity-induced glucose intolerance and hyperinsulinemia [42].

7.2. JNK1 Signaling Pathway. As seen in Figure 2, activation of JNK1 also results in inhibitory serine phosphorylation of IRS-1. In a manner similar to IKKβ, JNK1 can stimulate transcription of inflammatory genes in association with transcription factor activator protein 1 (AP1) [43]. Knockout (KO) of JNK1 in nonhematopoietic cells protected mice from HFD-induced insulin resistance, in part through decreased adiposity [40, 44]. By contrast, mice with JNK1 knocked out of hematopoietic cells (macrophage-specific cells) became obese on HFD, with hepatic steatosis and increased intramuscular triglyceride content, but were still protected against insulin resistance [44]. Protection against insulin resistance was conferred to these hematopoietic cell-specific KO mice by a decrease in ATM content and reduction in inflammatory pathway gene expression [44]. This experiment demonstrates

that obesity and tissue lipid burden may not be sufficient to cause insulin resistance. Without the inflammatory component, obesity does not lead to appreciably impaired insulin action as demonstrated in macrophage-specific IKKβ and JNK1-KO mice [42, 44].

7.3. Toll-Like Receptors and Lipid Mediators. ATMs exist in a lipid-rich milieu, and free fatty acids, abundant in that milieu, can have a variety of effects on macrophage inflammatory pathways. For instance, omega-3 fatty acids are anti-inflammatory, polyunsaturated fatty acids are weak or neutral, and saturated fatty acids are proinflammatory [45]. Toll-like pattern recognition receptors recognize molecules that are broadly shared by pathogens. Specifically, Toll-like receptor 4 (TLR4) is expressed on macrophages and recognizes not only LPS produced by gram-negative bacteria but also saturated fatty acids; both of these ligands can activate TLR4, resulting in activation of JNK and IKKβ. TLR4 expression is increased in obesity; when the gene encoding TLR4 was deleted, HFD mice were protected from insulin resistance and weight gain compared to controls [25, 46].

Lipid species appear to regulate inflammatory signaling in macrophages through non-TLR pathways as well. Overexpression of diacylglycerol transferase-1 (DGAT-1, which catalyzes the final stage in triglyceride synthesis) in macrophages protects against adipose macrophage infiltration, inflammation, and insulin resistance [47]. This suggests that the effects of triglycerides on macrophages may not be as inimical as the effects of the precursors of triglycerides, that is, fatty acids and diacylglycerols. Diacylglycerols in particular have been implicated in insulin resistance in liver and muscle [47]. In addition, ceramides have recently been shown to activate the nucleotide-binding domain, leucine-rich-containing family, and pyrin-domain-containing (NLRP) inflammasome [48]. NLRP activation ultimately leads to IL-1 β and IL-18 secretion, a response that is generally stimulated by “danger signals” of nonmicrobial origin [48]. Finally, omega-3 fatty acids have anti-inflammatory and antidiabetic effects in humans and mice. Activation of the omega-3 fatty acid receptor (GRP120) on macrophages and adipocytes reverses adipose inflammation and insulin resistance in obese mice [49].

7.4. Endoplasmic Reticulum (ER) Stress. Multiple proinflammatory sources can lead to activation of ATMs in obesity, including ER stress. ER stress can be stimulated by fatty acids, nutrient excess, improperly folded proteins, and regional areas of microhypoxia, all of which occur in obese adipose tissue. As obesity develops, protein biosynthetic pathways are upregulated, activating the unfolded protein response (UPR) in the ER [50]. The UPR comprises three main pathways controlled by ER membrane proteins: inositol-requiring enzyme (IRE)-1, protein kinase-like ER kinase (PERK), and activating transcription factor (ATF) α . All 3 branches of the UPR can directly engage inflammatory pathways through activation of IKK β and/or JNK signaling [50]. In summary, there are numerous mechanisms propagating inflammation in obesity, but most if not all appear linked to one of two final common pathways—JNK and IKK β —which lead to the end-result of insulin resistance.

8. Mechanisms to Counteract Inflammatory Signaling

8.1. Peroxisome Proliferator-Activated Receptors (PPARs). PPAR γ is a member of the nuclear receptor superfamily of ligand-dependent transcription factors that is predominantly expressed in adipose tissue and the intestines. PPAR γ is also highly expressed in macrophages and is a natural ligand to FFAs and eicosanoids. PPAR α and PPAR γ function as regulators of lipid metabolism and glucose homeostasis, respectively, and are targets for fatty acid oxidizing fibrates and insulin-sensitizing TZDs, respectively [51]. As seen in Figure 2, PPAR activators inhibit the activation of inflammatory response genes by interfering with the NF- κ B and AP1 signaling pathways, thereby promoting insulin sensitivity [51]. Consistent with this effect, Hevener et al. demonstrated that macrophage-specific PPAR γ KO induced glucose intolerance with skeletal muscle and hepatic insulin resistance in lean mice fed a normal diet [52]. This phenotype

was associated with increased expression of inflammatory markers and impaired insulin signaling in adipose tissue, muscle, and liver. Furthermore, insulin resistance became more severe in mice lacking macrophage PPAR γ following HFD feeding, and these mice were only partially responsive to TZD treatment [52]. Another member of the PPAR family, PPAR β/δ , is induced by IL-4 and IL-13 to promote alternative activation of macrophages. Myeloid-specific deletion of PPAR β/δ in mice has been shown to cause insulin resistance with increased adipocyte lipolysis and severe hepatosteatosis [53]. Thus PPARs function in an anti-inflammatory manner and promote M2 polarization.

9. Other Immune Cells in Adipose Tissue Contributing to Insulin Resistance

As obesity develops, enlarging adipocytes secrete chemokines that attract immune cells. Macrophages are amongst the earliest immune cells to infiltrate adipose tissue, as their numbers increase after one week of HFD [43]. Although macrophages are vital in innate and adaptive immunity, the immune response is a result of interactions between multiple cell types. Hence, regulatory T cells, CD8+ effector T cells, B cells, mast cells, and eosinophils within adipose tissue have also been implicated in the pathogenesis of obesity-related insulin resistance.

9.1. Lymphocytes. T cells are involved in adipose tissue inflammation and IR by modifying ATM numbers and affecting polarization states. T-helper (T_H) cells express the cell surface marker CD4 and comprise T_H1 cells (which produce proinflammatory cytokines) and T_H2 cells (which produce anti-inflammatory cytokines). Regulatory T cells (Tregs), another CD4(+) type, secrete anti-inflammatory signals, inhibit macrophage migration, and induce M2 polarization [54]. Feuerer et al. discovered that Tregs were highly enriched in the VAT of normal mice, but their numbers were strikingly reduced at this site in HFD-fed and genetically obese mice [54]. Furthermore, Treg deletion in mice induced acute elevations of TNF α and IL-6 transcripts in VAT, while inhibiting insulin signaling in muscle and liver [54]. In contrast to CD4(+) cells, T cells that express the surface antigen CD8 (known as effector or cytotoxic T cells) promote ATM accumulation and proinflammatory gene expression and are increased in number in obese adipose tissue [55]. Winer et al. highlighted the role of Tregs through analyses of T-cell deficient Rag-1(-/-) mice [56]. When placed on HFD, Rag-1(-/-) rodents were more insulin resistant than their controls. Adoptive transfer of CD4(+) to these rodents reversed the insulin resistant phenotype and abrogated VWAT inflammation; this was not observed with adoptive transfer of CD8(+) cells in Rag-1(-/-) rodents [56]. A critical unanswered question in regard to these profound effects of T cells is what in the milieu of adipose tissue activates them in the first place. It would be important to determine if the activator is a specific antigen or whether some form of completely antigen-independent T cell activation takes place.

B cells have also been ascribed functions in promoting VAT inflammation during weight gain. Winer et al. identified a VAT B-cell population that expanded with HFD feeding, with parallel increases in tissue IgM and IgG levels. Immunohistochemistry revealed deposits of these antibodies throughout VAT that mirrored the locations of CLS [57]. Mice lacking the immunoglobulin mu heavy chain (B-null mice) were protected from HFD-induced insulin resistance and displayed diminished VAT inflammation as indicated by lower numbers of M1-like macrophages and activated CD8(+) T cells. Strikingly, reconstitution of B-null mice with either wild-type (WT) B cells or serum immunoglobulins from WT HFD-fed mice was sufficient to restore the insulin resistant phenotype. Furthermore, adoptive B-cell transfer to lean WT mice induced insulin resistance [57]. These results demonstrate inflammatory pathways in which B cells and adaptive immunity play a role in insulin resistance. Again, the stimulus for B-cell activation, whether a specific antigen or otherwise, remains to be specified.

9.2. Mast Cells. Although mast cells are well known for their role in allergy and anaphylaxis, they seem to play a role in obesity as well. Altintas et al. found that subcutaneous fat of lean mice contained more mast cells, but fewer solitary macrophages and CLS than visceral fat [4]. With obesity, there was no significant change in mast cell density of subcutaneous fat, but there was a substantial increase in mast cell number in visceral fat. CLS became prevalent in visceral fat of obese mice, and their distribution paralleled that of mast cells. Immunofluorescence staining and confocal microscopy demonstrated that a subset of mast cells in adipose tissue contained and released preformed TNF- α as well [4]. In summary, subcutaneous fat differed from visceral fat by not only immune cell composition but also by having a lower prevalence of CLS both in lean and obese mice. The increase in mast cells in visceral fat of obese mice suggests a role in the pathogenesis of obesity and insulin resistance. Liu et al. discovered that in mice fed on a Western diet, genetically induced deficiency of mast cells or their pharmacological stabilization (with daily injections of disodium cromoglycate) reduces body weight gain and levels of inflammatory cytokines in serum and VAT, in conjunction with improved glucose homeostasis and energy expenditure [58].

9.3. Eosinophils. Similar to mast cells, eosinophils mediate allergic reactions in addition to combating parasites. Wu et al. demonstrated that eosinophils also participate in endorsing a M2-like ATM polarization state via IL-4 [59]. By analyzing VAT of mice on a normal chow diet, these investigators found that >90% of the IL-4-competent cells recovered were eosinophils. There was a reciprocal relationship between adipose eosinophil quantity and mouse weight. When placed on a HFD, those mice that were genetically deficient in eosinophils had increased body fat, impaired glucose tolerance, and insulin resistance in comparison to WT controls. Wu et al. also observed that mice on a HFD who were infected with helminths sustained a metabolic response characterized by decreased fasting glucose and improved insulin sensitivity

from the early postinfection phase and sustained up to 35 days following infection [59].

10. Conclusion

At a histological level, adipose tissue inflammation in obesity is associated with macrophage accumulation and development of CLS. At a cellular level, the resultant insulin resistance can be explained by activation of JNK1 and IKK β , two critical pathways mediating a range of inflammatory and stress mechanisms activated in obesity. It is interesting to note that many cellular and biochemical components of the immune system that normally protect the host from foreign pathogens, such as macrophages and TLRs, also play a pathologic role in obesity-related inflammation. Overall, adipose tissue inflammation in obesity demonstrates that the immune system and metabolism are highly integrated.

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Research Article

Maternal Docosahexaenoic Acid Increases Adiponectin and Normalizes IUGR-Induced Changes in Rat Adipose Deposition

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Intrauterine growth restriction (IUGR) predisposes to obesity and adipose dysfunction. We previously demonstrated IUGR-induced increased visceral adipose deposition and dysregulated expression of peroxisome proliferator activated receptor- γ 2 (PPAR γ 2) in male adolescent rats, prior to the onset of obesity. In other studies, activation of PPAR γ increases subcutaneous adiponectin expression and normalizes visceral adipose deposition. We hypothesized that maternal supplementation with docosahexaenoic acid (DHA), a PPAR γ agonist, would normalize IUGR adipose deposition in association with increased PPAR γ , adiponectin, and adiponectin receptor expression in subcutaneous adipose. To test these hypotheses, we used a well-characterized model of uteroplacental-insufficiency-(UPI-) induced IUGR in the rat with maternal DHA supplementation. Our primary findings were that maternal DHA supplementation during rat pregnancy and lactation (1) normalizes IUGR-induced changes in adipose deposition and visceral PPAR γ expression in male rats and (2) increases serum adiponectin, as well as adipose expression of adiponectin and adiponectin receptors in former IUGR rats. Our novel findings suggest that maternal DHA supplementation may normalize adipose dysfunction and promote adiponectin-induced improvements in metabolic function in IUGR.

1. Introduction

Intrauterine growth restriction (IUGR) predisposes to adult onset disease. The development of obesity following IUGR is well documented and results from adipose dysfunction [1–3]. While IUGR infants are smaller than their appropriately grown counterparts at birth, their rate of weight accretion is accelerated in childhood and they acquire more adipose tissue. An important concept is that IUGR adipose tissue is dysregulated before the onset of obesity [4, 5]. In addition to increased relative amounts of adipose tissue, adipose deposition in IUGR children favors formation of visceral adipose tissue (VAT) over subcutaneous adipose tissue (SAT) [6–8].

We previously demonstrated that IUGR increases the ratio of VAT to SAT in male adolescent rats, prior to the onset of obesity, with no effect in female rats [9]. The changes in adipose deposition in IUGR were accompanied by increased

expression of the adipogenic transcription factor peroxisome proliferator activated receptor- γ 2 (PPAR γ 2) in VAT, but not SAT, of male rats [9].

An important transcriptional target of PPAR γ is adiponectin [10, 11]. Adiponectin improves insulin sensitivity and normalizes adipose deposition [12]. When mice with excessive VAT deposition overexpress adiponectin, VAT is redistributed to SAT in association with improved metabolic parameters [12]. Interestingly, the same outcome occurs when obese mice are given a PPAR γ agonist [12]. PPAR γ agonists, such as the long chain fatty acid (FA) docosahexaenoic acid (DHA), improve metabolic disease in both human and animal models and increase PPAR γ mediated transcription of targets such as adiponectin [11, 13, 14].

The effects of adiponectin are imparted through one of its two receptors Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) [15]. AdipoR1 and AdipoR2 have been found to be decreased with obesity leading

to reduced adiponectin sensitivity [16]. Additionally, AdipoR1 and AdipoR2 are significantly decreased in adipose tissue of insulin resistant ob/ob mice when compared with control mice [16].

The effects of IUGR on adiponectin and adiponectin receptor expression in the rat are unknown. Also unknown are the effects of maternal DHA supplementation on adipose distribution, as well as expression of PPAR γ , adiponectin, and adiponectin receptors in the context of IUGR. Because DHA activates PPAR γ , we hypothesized that maternal DHA supplementation would normalize IUGR VAT and SAT levels in association with increased PPAR γ , adiponectin, AdipoR1 and AdipoR2 expression in SAT. To test these hypotheses, we used a well-characterized model of uteroplacental-insufficiency (UPI-) induced IUGR in the rat [9, 17, 18] and developed a model of maternal DHA supplementation in the context of the UPI-induced IUGR rat [19].

2. Materials and Methods

2.1. Animals. All procedures were approved by the University of Utah Animal Care Committee and are in accordance with the American Physiological Society's guiding principles [20]. IUGR was induced by uteroplacental insufficiency (UPI) in Sprague Dawley rats as previously described [9, 19, 21]. Briefly, on day 19 of gestation, pregnant Sprague-Dawley rats were anesthetized with intraperitoneal xylazine (8 mg/kg) and ketamine (40 mg/kg), and both uterine arteries ligated giving rise to IUGR pups. Control dams underwent identical anesthetic procedures. After recovery, rat dams had ad libitum access to food and water.

Maternal rats were allowed to deliver spontaneously at term; pups were weighed and litters randomly culled to six, to normalize postnatal nutrition. The pups remained with their mothers, feeding via lactation, until day 21 of postnatal life (d21). At d21, control and IUGR rat offspring underwent euthanasia using a sodium pentobarbital overdose (150 mg/kg), blood and serum were collected, and subcutaneous and retroperitoneal (a representative visceral depot) adipose was immediately harvested and flash frozen in liquid nitrogen.

2.2. Maternal DHA Supplementation. DHA was administered via a custom diet [19]. The diet, based on Harlan Teklad 8640 standard rodent diet (TD.8640, Harlan-Teklad, WI), substitutes 1% of the soybean oil in the standard chow with 1% purified DHA (cis-docosahexaenoic acid, no. U-84-A, Nu-Chek Prep, MN). The resulting diet (here called 1% DHA diet) contains the same macronutrient content as standard rodent chow (21.8% protein, 40.8% carbohydrate, and 5.4% fat, with a resulting caloric density of 3 Kcal/g). DHA at 1% was chosen based on our previous studies demonstrating that maternal supplementation with 1% DHA was sufficient to increase pup DHA levels and to be associated with alterations in PPAR γ in other tissues [19].

The pregnant rats were pair-fed regular diet or 1% DHA diet from E13 and through lactation. The continuation of the maternal diet is important to this study as the fatty

acid composition of the maternal milk reflects the maternal dietary fatty acid profile [22, 23]. Each group (Control, IUGR and DHA-IUGR) consisted of 6 male and 6 female pups derived from different litters.

2.3. Magnetic Resonance Imaging. Magnetic resonance imaging (MRI) experiments were conducted using a Bruker Biospec 70/30 instrument (Billerica, MA) and a 72 mm-diameter birdcage radiofrequency (RF) transmit-receive resonator as previously described [9]. ImageJ macros were created to automate the process of image analysis while still allowing manual intervention at key steps that were less amenable to automation [9, 24].

2.4. Serum Adiponectin Quantification. Serum adiponectin was quantified using an enzyme linked immunosorbent assay (ELISA) (Alpco Diagnostics, NH, (44-ADPRT-E01)) according to the manufacturer's instructions.

2.5. Real-Time RT-PCR. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to evaluate mRNA levels in subcutaneous and visceral adipose tissue of adiponectin and adiponectin receptors as previously described [9, 19, 25]. The following Assay-on-demand primer/probe sets were used: PPAR γ 2-Rn00440940.m1, adiponectin-Rn00595250.m1, AdipoR1-Rn01483784.m1, and AdipoR2-Rn01463173.m1 (Applied Biosystems, CA, USA). GAPDH was used as an internal control (GAPDH primer and probe sequences; Forward: CAAGATGGTGAAGGTCG-GTGT; Reverse: CAAGAGAAGGCAGCCCTGGT; Probe: GCGTCCGATACGGCCAAATCCG).

2.6. Protein Analysis. Adipose tissue levels of PPAR γ 2, AdoR1, and AdoR2 protein were quantified using immunoblotting as previously described [9, 19]. The following primary antibodies were used: PPAR γ (H-100, sc-7196, Santa Cruz Biotechnology), AdipoR1 (Santa Cruz Biotechnology, CA, sc-46748), and AdipoR2 (Alpha Diagnostic International, TX, ApidoR21-A).

2.7. Statistics. Data are presented as IUGR or DHA-IUGR relative to sex-matched controls \pm SEM. Statistical significance was determined using ANOVA using the StatView 5 software package (SAS Institute, Inc.). $P \leq 0.05$ was considered significant.

3. Results

3.1. Body Weights. Control rat dams supplemented with DHA did not differ in body weight from control rat dams fed a regular diet. Similarly, IUGR rat dams supplemented with DHA did not differ in body weight from IUGR rat dams fed a regular diet. DHA diet also did not affect newborn pup weights for pups from either control dams or IUGR dams (Table 1). Consistent with previously published findings [9], IUGR pups weighed significantly less than control pups through d21. DHA-IUGR significantly increased body weight

TABLE 1: Maternal DHA supplementation did not alter control or IUGR dam or pup weights relative to regular diet (mean g \pm SD).

	Maternal weight		Pup weight	
	Control	IUGR	Control	IUGR
Regular diet	383 \pm 34	294 \pm 12	6.4 \pm 0.6	5.6 \pm 0.2
DHA diet	349 \pm 18	295 \pm 16	6.3 \pm 0.3	5.5 \pm 0.6

of male pups and normalized weight of female pups relative to regular diet controls (Figure 1(a)).

3.2. Adipose Tissue Distribution. Relative levels of VAT (retroperitoneal) and SAT were quantified from MRI images in male and female control, IUGR and DHA-IUGR rats at d21. Consistent with our previously published study, in male rats, IUGR increased levels of both SAT and VAT relative to regular diet control ($P \leq 0.05$). In female rats, IUGR did not significantly alter levels of VAT or SAT (Figure 1(b)). DHA-IUGR did not significantly alter levels of VAT or SAT in either male or female rats relative to regular diet controls (Figure 1(b)).

3.3. PPAR γ 2 mRNA and Protein. Consistent with our previous study, PPAR γ 2 mRNA was unchanged in SAT and increased in VAT of male rats ($P < 0.05$) relative to male control rats. PPAR γ 2 mRNA was unchanged in female SAT or VAT relative to female control rats. PPAR γ 2 protein abundance was similarly increased in the VAT of male rats relative to male control. PPAR γ 2 protein abundance was unchanged in SAT or VAT of IUGR females. DHA-IUGR significantly increased PPAR γ 2 mRNA in SAT of male ($P \leq 0.05$) and female ($P < 0.05$) rats relative to sex-matched controls. DHA-IUGR did not significantly alter PPAR γ 2 protein abundance in male or female SAT or VAT (Figure 2).

3.4. Serum Adiponectin. IUGR did not affect serum adiponectin levels in either male or female rats relative to sex-matched controls. However, DHA-IUGR significantly increased serum adiponectin levels in male and female ($P < 0.05$) rats relative to sex-matched regular diet controls (Figure 3).

3.5. Adiponectin mRNA. Adiponectin mRNA was unaffected by IUGR in SAT and VAT of male or female rats. DHA-IUGR significantly increased adiponectin mRNA in SAT of male ($P \leq 0.05$) and female ($P < 0.05$) rats relative to regular diet controls. DHA-IUGR did not affect VAT levels of adiponectin mRNA in male or female rats (Figure 4).

3.6. AdipoR1 mRNA and Protein. In male rats, IUGR significantly increased AdipoR1 mRNA in VAT ($P \leq 0.05$) relative to male control VAT. IUGR did not alter AdipoR1 mRNA in SAT and VAT of female rats. AdipoR1 protein abundance was also unchanged by IUGR in SAT and VAT of male or female rats relative to sex-matched controls. DHA-IUGR significantly increased AdipoR1 mRNA in male SAT ($P \leq 0.05$) relative to male control SAT. DHA-IUGR did

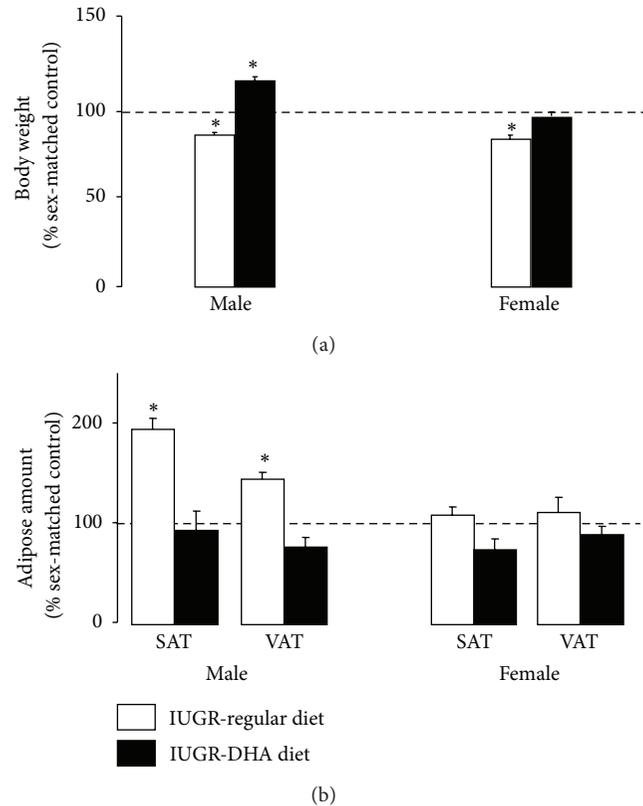


FIGURE 1: IUGR and DHA-IUGR body weight and adipose deposition. (a) IUGR decreased body weights of male and female adolescent day 21 rats. DHA-IUGR increased male body weight and normalized female body weight compared to controls. (b) Quantification of MRI images. IUGR increased SAT and VAT in male rats. DHA-IUGR normalized SAT and VAT levels in male rats. SAT and VAT deposition was not affected by IUGR or DHA-IUGR in female rats. Results are IUGR (white bars) or DHA-IUGR (black bars) relative to sex-matched controls (represented by the dotted line). Errors are SEM. SAT is subcutaneous adipose tissue, VAT is visceral adipose tissue, $*P \leq 0.05$.

not affect AdipoR1 mRNA levels of female rats in VAT or SAT. DHA-IUGR significantly increased AdipoR1 protein levels in female SAT ($P \leq 0.05$), with a trend towards significance in male SAT ($P = 0.06$). Levels of AdipoR1 protein were undetectable in DHA-IUGR rat male and female VAT (Figure 5).

3.7. AdipoR2 mRNA and Protein. In male rats, IUGR significantly increased AdipoR2 mRNA in SAT ($P < 0.05$) relative to male control SAT. AdipoR2 mRNA was unaffected by IUGR in SAT or VAT of female rats. AdipoR2 protein was unaffected by IUGR in SAT and VAT of male or female rats. DHA-IUGR increased AdipoR2 mRNA in male ($P \leq 0.05$) and female ($P \leq 0.05$) SAT. DHA-IUGR AdipoR2 protein abundance was increased in male ($P < 0.05$) and female ($P < 0.05$) SAT (Figure 6).

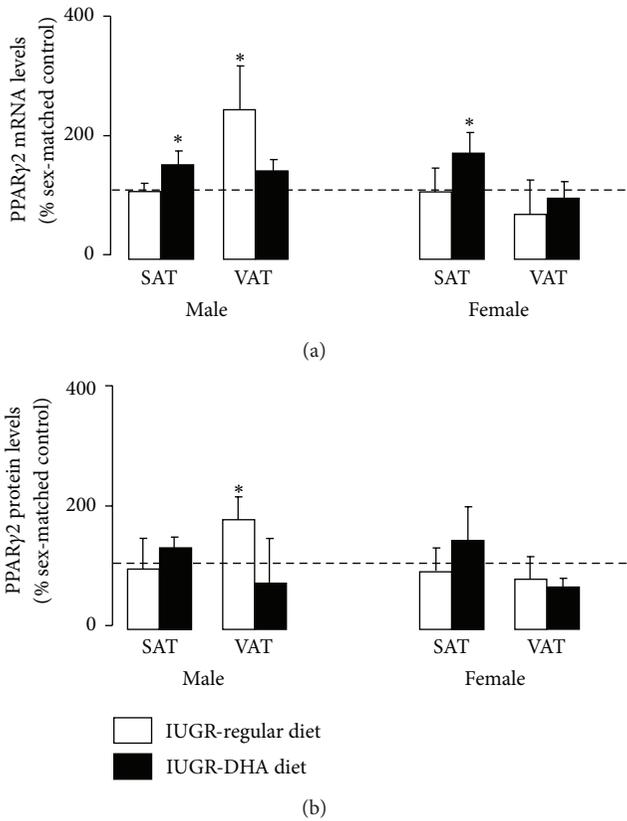


FIGURE 2: PPAR γ 2 mRNA and protein levels. IUGR increases VAT PPAR γ 2 mRNA and protein levels in male rats. DHA-IUGR normalized male VAT PPAR γ 2 levels and increased SAT PPAR γ 2 mRNA levels male and female rats. Results are IUGR (white bars) or DHA-IUGR (black bars) relative to sex-matched controls (represented by the dotted line). Errors are SEM. SAT is subcutaneous adipose tissue, and VAT is visceral adipose tissue, * $P \leq 0.05$.

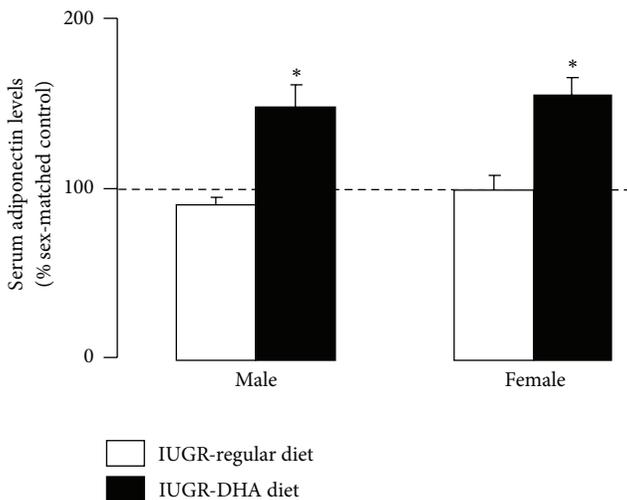


FIGURE 3: Serum adiponectin levels. DHA-IUGR increased serum adiponectin levels in male and female rats. Results are IUGR (white bars) or DHA-IUGR (black bars) relative to sex-matched controls (represented by the dotted line). Errors are SEM. * $P \leq 0.05$.

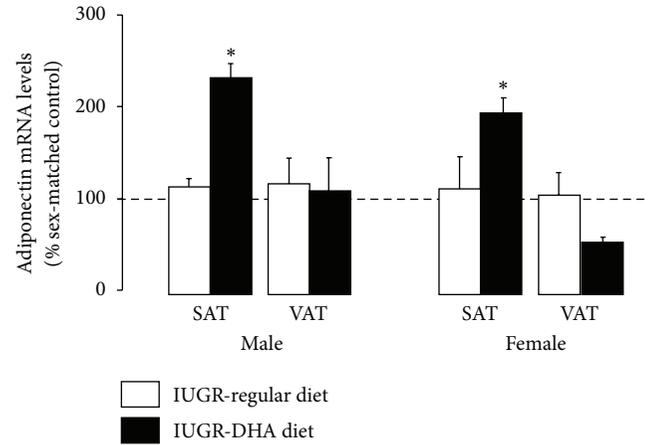


FIGURE 4: Adiponectin mRNA levels. DHA-IUGR increased SAT adiponectin mRNA levels in male and female rats. Results are IUGR (white bars) or DHA-IUGR (black bars) relative to sex-matched controls (represented by the dotted line). Errors are SEM. SAT is subcutaneous adipose tissue, and VAT is visceral adipose tissue * $P \leq 0.05$.

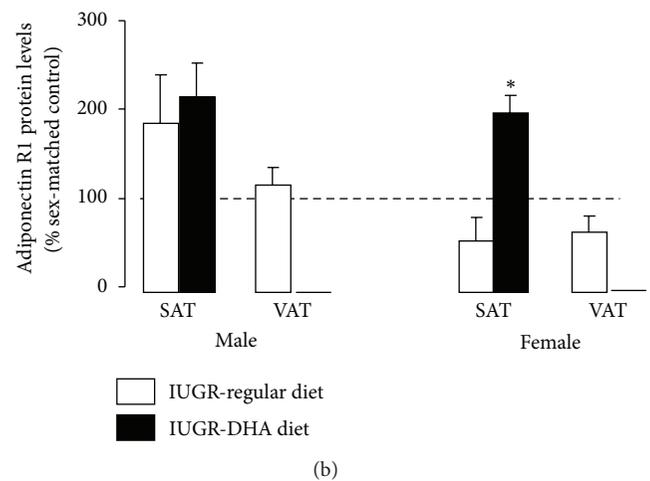
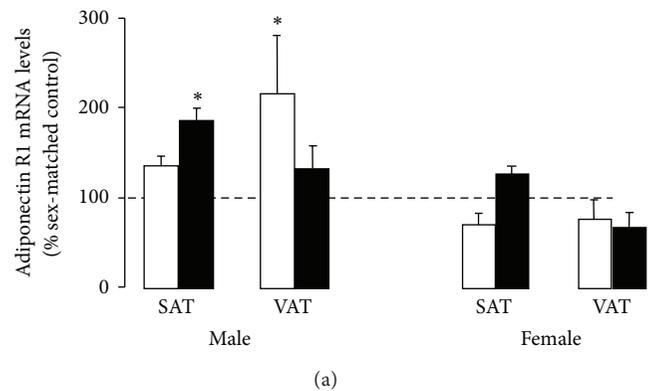


FIGURE 5: AdipoR1 mRNA and protein levels. IUGR increases VAT AdipoR1 mRNA levels in male rats. DHA-IUGR normalized male VAT AdipoR1 mRNA levels and increased SAT AdipoR1 levels male and female rats. Results are IUGR (white bars) or DHA-IUGR (black bars) relative to sex-matched controls (represented by the dotted line). Errors are SEM. SAT is subcutaneous adipose tissue, and VAT is visceral adipose tissue. * $P \leq 0.05$.

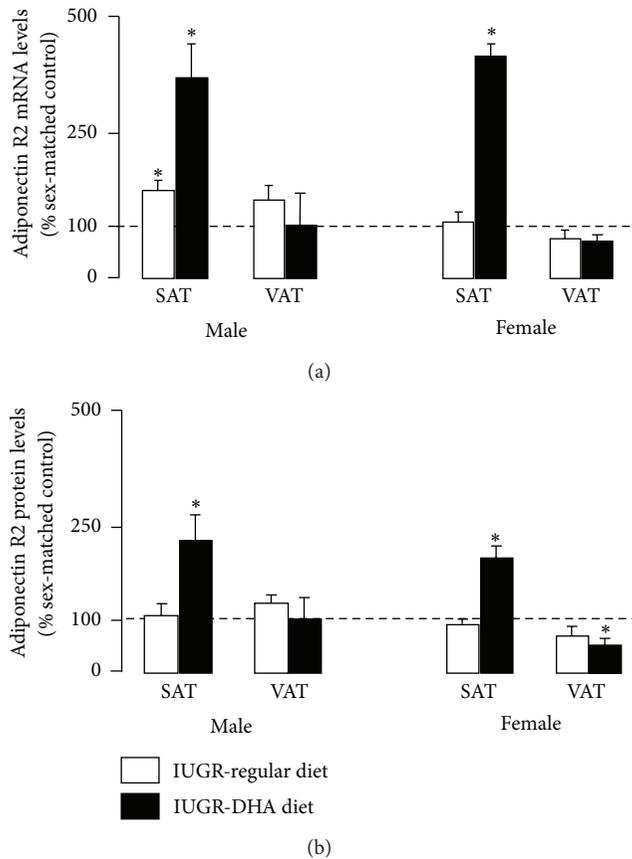


FIGURE 6: AdipoR2 mRNA and protein levels. IUGR increases SAT AdipoR2 mRNA levels in male rats. DHA-IUGR increased SAT AdipoR2 mRNA and protein levels in male and female rats. Results are IUGR (white bars) or DHA-IUGR (black bars) relative to sex-matched controls (represented by the dotted line). Errors are SEM. SAT is subcutaneous adipose tissue, and VAT is visceral adipose tissue, * $P \leq 0.05$.

4. Discussion

Important findings of our study are twofold. Firstly, maternal DHA supplementation during rat pregnancy and lactation normalizes IUGR-induced changes in adipose deposition and visceral PPAR γ expression in male rats. Secondly, maternal DHA supplementation increases serum adiponectin, as well as adipose expression of adiponectin and adiponectin receptors in former IUGR rats. Our novel findings suggest that maternal DHA supplementation may normalize adipose dysfunction and promote adiponectin-induced improvements in metabolic function in IUGR.

Individuals born IUGR develop adult obesity, with preferential VAT deposition, and insulin resistance [8, 26, 27]. Obesity, preferential VAT deposition, and insulin resistance have been demonstrated in IUGR animal models by our group and others [9, 28–31], often with a sex-specific bias [9, 29, 31]. A common theme amongst IUGR animal models is dysfunctional adipose gene expression that occurs prior to the onset of overt obesity. Our previous observation of increased VAT deposition in adolescent IUGR male rats

is consistent with the subsequent development of insulin resistance in this model. This is because VAT and SAT contribute differently to the development of insulin resistance, with VAT being detrimental and SAT potentially conferring protective roles [12, 32, 33]. Normalization of VAT levels in IUGR males following maternal DHA supplementation may be metabolically protective in IUGR. Interestingly, while VAT levels normalized and SAT levels did not increase in IUGR-DHA male rat pups, DHA-IUGR male body weight was increased in our study. This may represent alterations in lean body mass accretion in DHA-IUGR pups.

Supplementation with DHA is physiologically relevant in IUGR. A maternal plasma FA profile low in DHA has been shown to be associated with small for gestational age (SGA) and preterm birth. Additionally, maternal supplementation with DHA during gestation is associated with longer gestation duration [34–36]. In our IUGR rat model, serum DHA is decreased in male neonatal rat pups compared to control, with no significant difference seen in females [19]. The addition of DHA to the maternal diet increases serum DHA levels in male and female DHA-IUGR relative to control [19]. In our model, improved DHA status normalizes VAT PPAR γ 2 levels in male IUGR rats and increases SAT PPAR γ 2 levels in both male and female IUGR rats. These findings are consistent with results of PPAR γ activation in obese mice. In ob/ob mice, the synthetic PPAR γ agonist, Rosiglitazone, results in a redistribution of adipose from VAT to SAT stores, with a concomitant resolution of metabolic disturbances [12]. It is important to note however, that DHA may also help normalize adipose physiology by contributing to other mechanisms, including inhibition of inflammatory pathways [37, 38].

There is evidence that adiponectin may be the link between SAT PPAR γ activation and subsequent ablation of metabolic disturbances. Adiponectin expression and release are higher in SAT compared with VAT [39]. When systemically activated, SAT PPAR γ prevents adipocyte hypertrophy by increasing the number of small adipocytes which increases adiponectin levels [40]. In ob/ob mice, overexpression of adiponectin produces a similar effect to that of PPAR γ activation, with adipose redistribution from VAT to SAT and normalization of metabolic disturbances [12]. Adiponectin deficient mice have increased insulin resistance [41, 42]. Interestingly, obese humans express significantly lower levels of adiponectin even though adiponectin is expressed solely by adipose tissue [43]. Of note, children who are born IUGR also have lower circulating adiponectin at birth [43, 44]. Increased serum adiponectin, especially in individuals who have a predisposition to be insulin resistant, such as those born IUGR, may improve insulin sensitivity.

In addition to increased serum adiponectin, maternal DHA supplementation increased expression of AdipoR1 and AdipoR2 in SAT in our study. AdipoR1 and AdipoR2 play physiologically important roles in the regulation of insulin sensitivity and glucose metabolism *in vivo* [45]. Obese, insulin resistant mice have decreased expression of adiponectin receptors in adipose tissue [23]. The decreased expression of adiponectin receptors is associated with a reduction in the insulin-sensitizing effects of adiponectin.

Increasing AdipoR1 and/or AdipoR2 may enhance adiponectin binding capacities in adipose cells and in turn may lead to an increase in the adiponectin effects, even without change in adiponectin levels [46]. Overexpression of AdipoR1 and AdipoR2 in ob/ob mice normalizes insulin signaling [47]. Therefore, DHA-induced increases in both adiponectin as well as its receptors observed in this study may lead to the greatest degree of insulin sensitizing through adiponectin signaling mechanisms.

Our study is not without limitations. First, while we assessed the expression of adiponectin and AdipoR's in adipose tissue, we did not assess adiponectin signaling. An understanding of the downstream consequences of AdipoR activation in IUGR with and without DHA supplementation is important and warrants further investigation. Secondly, while we demonstrated improvement in adipose deposition and increased adiponectin with DHA supplementation in IUGR, we did not assess the effect of DHA supplementation on the development of insulin resistance in this model. Future studies are required to assess the effects of maternal DHA supplementation during gestation and lactation on the development of insulin resistance in IUGR rats. We also did not differentiate between prenatal DHA effects and postnatal DHA effects. Determining the minimum window in which DHA may be effective will be important to enhance the translational relevance of our study.

In conclusion, maternal DHA supplementation increases adiponectin and adiponectin receptor expression in SAT of IUGR rats. We speculate that increased adiponectin production may improve insulin sensitivity in IUGR rats. Our study suggests that early DHA supplementation may provide a means of tempering adipose dysregulation and subsequent metabolic disturbances in IUGR individuals.

Conflict of Interests

None of the authors have any conflict of interests.

Acknowledgments

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Clinical Study

Metabolic Risk Susceptibility in Men Is Partially Related to Adiponectin/Leptin Ratio

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Background. High adiponectin/leptin ratio may be protective from metabolic risks imparted by high triglyceride, low HDL, and insulin resistance. **Methods.** This cross-sectional study examines plasma adipokine levels in 428 adult men who were subgrouped according to low ($<6.5 \mu\text{g/mL}$) and high ($\geq 6.5 \mu\text{g/mL}$) adiponectin levels or a low or high ratio of adiponectin/leptin. **Results.** Men with high adiponectin/leptin ratio had lower plasma triglyceride and higher HDL cholesterol than those with low ratio. Similarly, those with high adiponectin/leptin ratio had lower TG/HDL cholesterol ratio and HOMA2-IR than those with low ratio. In contrast, levels of adiponectin or the ratio of adiponectin/leptin did not associate with systolic blood pressure. But the ratio of adiponectin/leptin decreased progressively with the increase in the number of risk factors for metabolic syndrome. **Conclusion.** Adipokine levels may reflect adipose tissue triglyceride storage capacity and insulin sensitivity. Leptin is an index of fat mass, and adiponectin is a biomarker of triglyceride metabolism and insulin sensitivity. Men with high adiponectin/leptin ratios have better triglyceride profile and insulin sensitivity than men with a low ratio regardless of waist girth.

1. Introduction

Excess abdominal body fat is implicated in the etiology of the metabolic syndrome, a cluster of risk factors for cardiovascular disease and for type 2 diabetes mellitus. The risk factors include dyslipidemia (high triglyceride and/or low HDL cholesterol), insulin resistance, and hypertension [1]. Abdominal obesity is assessed by waist girth, and several cut points of high-risk waist girth have been recommended to identify at-risk individuals based on gender and ethnicity [2]. Reaching a consensus on a sex-specific, global definition of high-risk waist girth has proved to be challenging [3]. However, a global cut point may be impractical because individuals vary in susceptibility to obesity-induced risk for metabolic syndrome. An alternative to using waist girth is to identify biomarkers that are causally related to the metabolic risks and that reflect a function of adipose tissue.

Two adipokines, leptin and adiponectin, may be risk markers of fat-induced dyslipidemia and insulin resistance.

Both adipokines are reportedly associated with risk for type 2 diabetes and with cardiovascular disease. Plasma levels of leptin correlate positively with total body fat [4–6] and with adipocyte number in men [7]. In addition, individuals at high risk seemingly have high levels of plasma leptin [8]. The levels are directly proportional to secretion rates of the adipokine by adipose tissue [9], which is primarily produced in subcutaneous tissue [10]. Adiponectin, in turn, is an insulin-sensitizing adipokine as well as an anti-inflammatory and antiatherogenic hormone. In obese subjects, levels of plasma adiponectin are reduced suggesting an abnormality in adipose tissue function. Furthermore, mutations in the gene encoding for adiponectin are associated with type 2 diabetes mellitus and features of metabolic syndrome including hypertension, dyslipidemia, and atherosclerosis [11]. It is still unclear whether adiponectin production differs between subcutaneous and visceral adipose tissue. However, it has been suggested that adiponectin levels in plasma are inversely

TABLE 1: Subject demography and baseline characteristics.

	Nonobese	Overweight	Obese
Waist girth category	<90 cm	90–101 cm	≥102 cm
Number of men (% of total)	55 (13)	147 (34)	226 (53)
Age (±SD) (years)	53.0 (8.9)	54.1 (10.2)	55.4 (9.6)
Body mass index (kg/m ²)	24.1 (2.3)	27.9 (2.5) ^b	33.6 (4.1) ^a
Waist girth (cm)	85.3 (3.6)	95.9 (2.9) ^b	112.8 (9.3) ^a
Systolic blood pressure (Hg mm)	132.1 (17.9) ^c	138.3 (16.7)	142.5 (16.7)
Diastolic blood pressure (Hg mm)	79.1 (12.9) ^c	82.3 (9.7)	83.0 (8.9)
Hemoglobin A1c (%), Median (IQ)*	5.4 (.4)	5.4 (.5)	5.5 (.6)
Glucose (mg/dL)	93.3 (9.3) ^d	94.9 (10.0)	97.0 (11.3)
HOMA2-IR median (IQ)	1.16 (.98)	1.57 (.77)	2.13 (1.53) ^a
HOMA2%β, median (IQ)	97.5 (51.9)	119.8 (47.4)	139.9 (70.6)
HOMA2%S, median (IQ)	86.5 (101.7)	63.7 (33.3) ^b	46.9 (36.4) ^a
Adiponectin μg/mL, median (IQ)	8.9 (87.6) ^c	7.7 (4.8)	7.5 (4.7)
Leptin ng/mL, median (IQ)	2.3 (2.2)	5.2 (3.9) ^b	10.4 (7.5) ^a
Triglyceride, median (IQ)	110 (68)	153 (104) ^b	186 (92) ^a
HDL cholesterol mg/dL	47 (15)	39 (15) ^b	37 (9) ^a
Non-HDL cholesterol mg/dL	147 (44)	166 (43)	165 (47)
Total Apo B mg/dL	116 (29)	126 (28) ^b	129 (26) ^a
Hypertension (%)	52.6	75.5	88.8
Smokers (%)	.0	3.7	1.5

^aSignificantly different from non-obese and overweight ($P < .02$); ^bsignificantly different from non-obese ($P < .02$); ^csignificantly different from overweight and from obese ($P < .02$); ^dsignificantly different from obese ($P < .02$); *IQ: interquartile.

correlated with visceral adiposity [12] and are positively correlated with lower extremity fat [13].

In the current study, levels of plasma adiponectin and leptin were examined in men with varying degrees of obesity that had marked interindividual variation in plasma triglycerides and insulin sensitivity. The question addressed was whether adiponectin alone or in combination with leptin had an effect on dyslipidemia and insulin resistance.

2. Materials and Methods

Four hundred and seventy-two adult men from the Veterans Affairs Medical Center (VAMC) at Dallas were recruited into a cross-sectional study designed to quantify risk factors for cardiovascular disease [14]. Several individuals that were enrolled had stable hypertension, a few (9% of total) also had type 2 diabetes mellitus treated with hypoglycemic agents consisting mostly of metformin and/or glyburide, and very few subjects (4%) had history of coronary heart disease (CHD). For the current analyses, men with type 2 diabetes mellitus were excluded. Data from 428 subjects was analyzed, and summaries are shown in Table 1 and Figures 2, 3, and 4.

The racial composition of enrolled individuals was representative of research participants at the VAMC; that is, 72% were non-Hispanic White, 21% were non-Hispanic Black, 6% were Hispanic, and 1% comprised other groups. Subjects were seen in the clinical research unit, and they had clinical assessment and anthropometry. Fasting blood was drawn for measurement of plasma lipids, lipoproteins, glucose, hemoglobin A1c, apolipoprotein B, and adipokines.

All study volunteers gave written informed consent to participate in the study that had been approved by the Institutional Research Board for Investigation in Humans.

3. Laboratory Measurements

Plasma total cholesterol, triglyceride, lipoprotein cholesterol, and apolipoprotein B were measured as previously described [14]. Levels of plasma insulin, leptin, and total adiponectin were measured by radioimmunoassay as detailed before [15].

4. Biostatistics

Data are summarized as means ± SD or medians (interquartiles (IQ)), and comparisons of means were done by analysis of variance (ANOVA) with the Bonferroni adjustments for multiplicity of testing as needed. Some variables were positively skewed and were log transformed before parametric analyses (triglyceride, leptin, adiponectin, and HOMA2-IR). The Kruskal-Wallis rank test was carried out for comparisons of mean rank adiponectin/leptin ratios as a function of a number of risk factors for metabolic syndrome. Adiponectin and the ratio of adiponectin to leptin were also employed to create dichotomous groups of insulin resistance and dyslipidemia risk factors. The cut points were the median for adiponectin as previously detailed [13] and the median for the adiponectin/leptin ratios for each waist girth category. Accordingly, waist girth category <90 cm had a median ratio of 3.7, waist girth 90–101 cm had a median ratio of 1.39, and waist girth >90 cm category had a median ratio of .69. An SAS

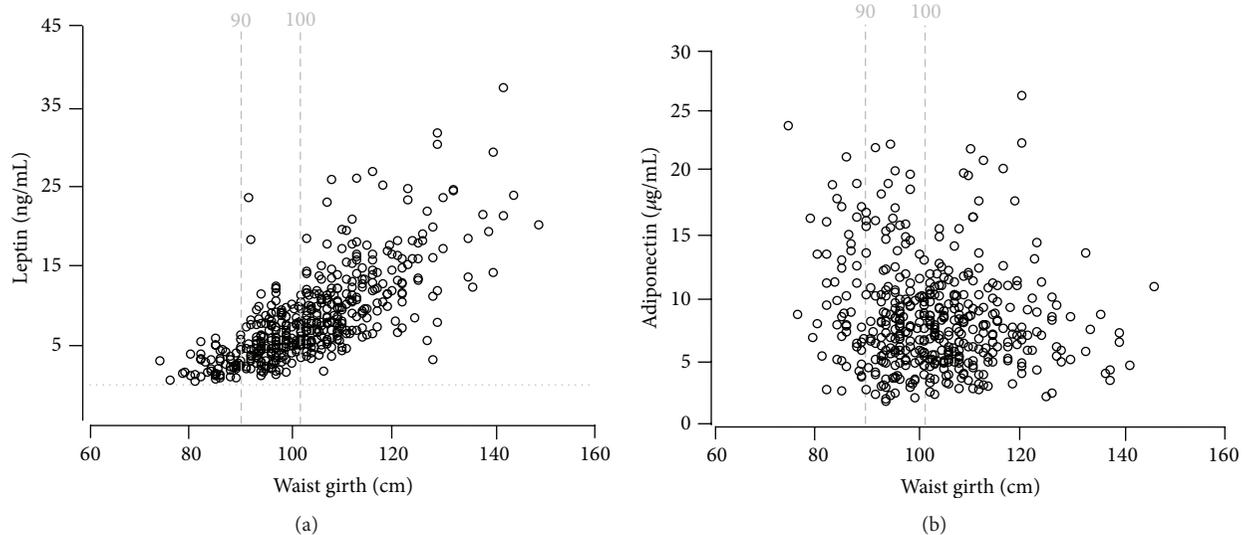


FIGURE 1: Scattered plots of leptin (a) and adiponectin (b) as a function of waist girth. There was a trend for a linear association of leptin to waist girth ($R^2 = .56$; $P < .001$). Subjects with waist girths below 90 cm consistently had the lowest leptin levels. More interindividual variations were noted in the leptin levels of subjects in waist girth in the range of 90 to 101 cm. In contrast to leptin, adiponectin was not linearly associated with waist girth (b). There was a striking interindividual variation in adiponectin levels at each waist girth subcategory, that is, nonobese, overweight, and obese.

version of Stat View was employed. HOMA2-IR calculator described by Levy et al. [16] was used.

5. Results

5.1. Characteristics of Study Subgroups. Men were subgrouped according to waist girth because this anthropometric measure is recommended for assessment of metabolic risk imparted by central obesity [1–3]. The waist girth cut points coincide with BMI cut points for nonobese (BMI <25 kg/m² or waist girth <90 cm), overweight (BMI 25 to 29.9 kg/m² or waist girth 90 to 101 cm), and obese men (BMI ≥ 30 kg/m² or waist girth ≥ 102 cm) [17]. Accordingly, 13% of the men were nonobese, 34% were overweight, and 53% were obese. The men were of similar age across the waist girth subgroups (Table 1).

Several measures of insulin resistance were different among the subgroups. First, overweight and obese men had higher fasting glucose concentrations and tended to be more insulin resistant compared to the nonobese group as shown by the HOMA2-IR levels (Table 1). Overweight and obese men had a lower insulin sensitivity (HOMA2%S) compared with nonobese, but there were no significant differences in the steady-state beta-cell function estimated by HOMA2% β . Levels of hemoglobin A1c were similar among the subgroups.

Overweight and obese men had higher triglyceride levels, reduced HDL cholesterol, and increased apolipoprotein B levels. However, levels of non-HDL cholesterol (i.e., VLDL + LDL) were not significantly different.

Nonobese men had lower blood pressure than overweight or obese men. The prevalence of hypertension ($\geq 130/85$ Hgmm or stable on antihypertensive medication)

was high among the three subgroups; smoking prevalence was relatively low (Table 1).

5.2. Interindividual Variation in Adipokines. Scattered plots of leptin versus waist girth (Figure 1(a)) and adiponectin versus waist girth (Figure 1(b)) were examined. There was a linear association of leptin to waist girth ($\text{Ln leptin} = -19.267 + 4.569$ (waist girth); $R^2 = .56$; $P < .001$). Lean men consistently had the lowest leptin levels (<5 ng/mL). More interindividual variations were noted in the leptin levels of overweight and obese men. In contrast to leptin, adiponectin was not linearly associated with waist girth (Figure 1(b)). Instead, there was a striking interindividual variation in adiponectin levels ranging from very low to high levels at each waist girth subcategory (nonobese, overweight, and obese individuals).

5.3. Adipokine Ratios and Metabolic Risk Factors. Levels of plasma triglyceride, HDL cholesterol, ratio of triglyceride/HDL cholesterol (C), HOMA2-IR, and systolic blood pressure were examined according to cut-off points for adiponectin (<6.5 ng/mL designated as low levels of adiponectin and ≥ 6.5 ng/mL designated as high levels) and according to adiponectin/leptin categories. The cut-off point for the ratio of adiponectin/leptin was the median ratio for each waist girth category as indicated in the statistical section.

Levels of plasma triglyceride in nonobese, overweight, and obese men grouped according to low or high adiponectin levels were compared (Figure 2(a)). Overweight men with high adiponectin levels had significantly lower levels of plasma triglyceride ($^a P < .02$) compared with those men with

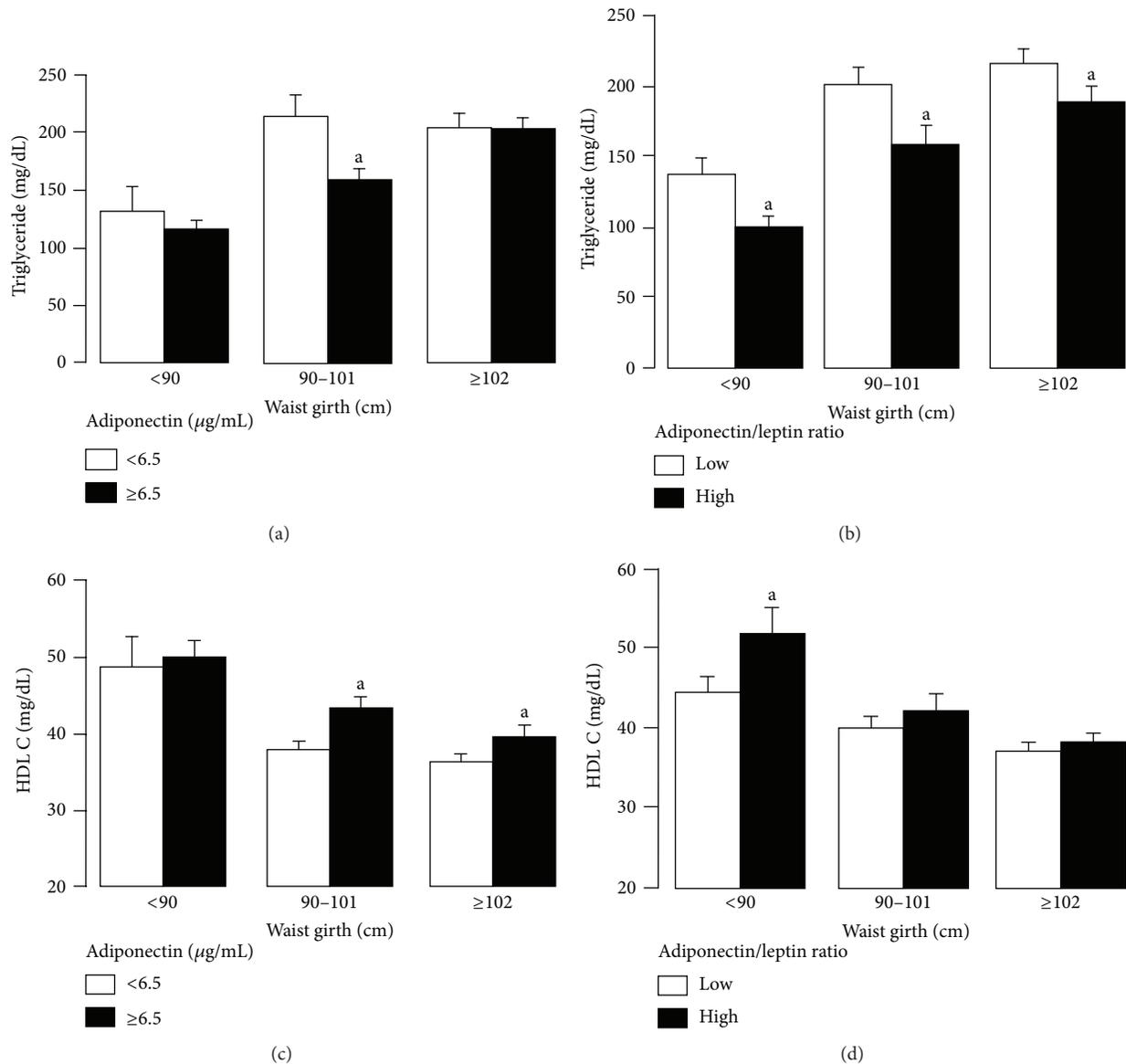


FIGURE 2: Levels of plasma triglyceride in nonobese (waist girth <90 cm), overweight (waist girth 90 to 101 cm), and obese (≥ 102 cm) grouped according to low (<6.5 ng/mL) or high (≥ 6.5 ng/mL) adiponectin. Overweight men with high adiponectin levels have significantly ($^aP < .02$) lower levels of plasma triglyceride compared with those with low adiponectin levels (a). However, after adjustment of adiponectin levels for leptin (ratio of adiponectin/leptin), levels of plasma triglycerides were significantly ($^aP < .02$) lower in those with a high ratio of adiponectin/leptin, regardless of waist category (nonobese, overweight, or obese) (b). Similar analyses are shown for HDL C. Levels of plasma HDL C were significantly ($^aP < .02$) higher in overweight and obese men having a high adiponectin level (c). However, HDL cholesterol levels were significantly higher ($^aP < .02$) in nonobese men after grouping the subjects according to a low or high ratio of adiponectin/leptin (d).

low adiponectin levels. Levels of plasma triglyceride for each obesity category also were compared between men with low or high ratios of adiponectin/leptin (Figure 2(b)). Levels of plasma triglycerides were significantly lower ($^aP < .02$) in individuals with a high ratio of adiponectin/leptin, regardless of waist category (nonobese, overweight, or obese).

HDL cholesterol (C) levels were significantly higher in overweight and obese men ($^aP < .02$) that had a high level of adiponectin compared with those that had a low level of

(Figure 2(c)). However, HDL cholesterol levels were significantly higher in nonobese men ($^aP < .02$) after grouping the subjects according to a low or high ratio of adiponectin/leptin (Figure 2(d)).

Ratio of triglyceride/HDL cholesterol (C) was lower in nonobese ($^bP < .05$) and overweight ($^aP < .02$) men who had a high level of plasma adiponectin (Figure 3(a)). In contrast, the ratio of plasma triglyceride/HDL C was lower ($^aP < .02$) in nonobese, overweight, and obese subjects with a high ratio

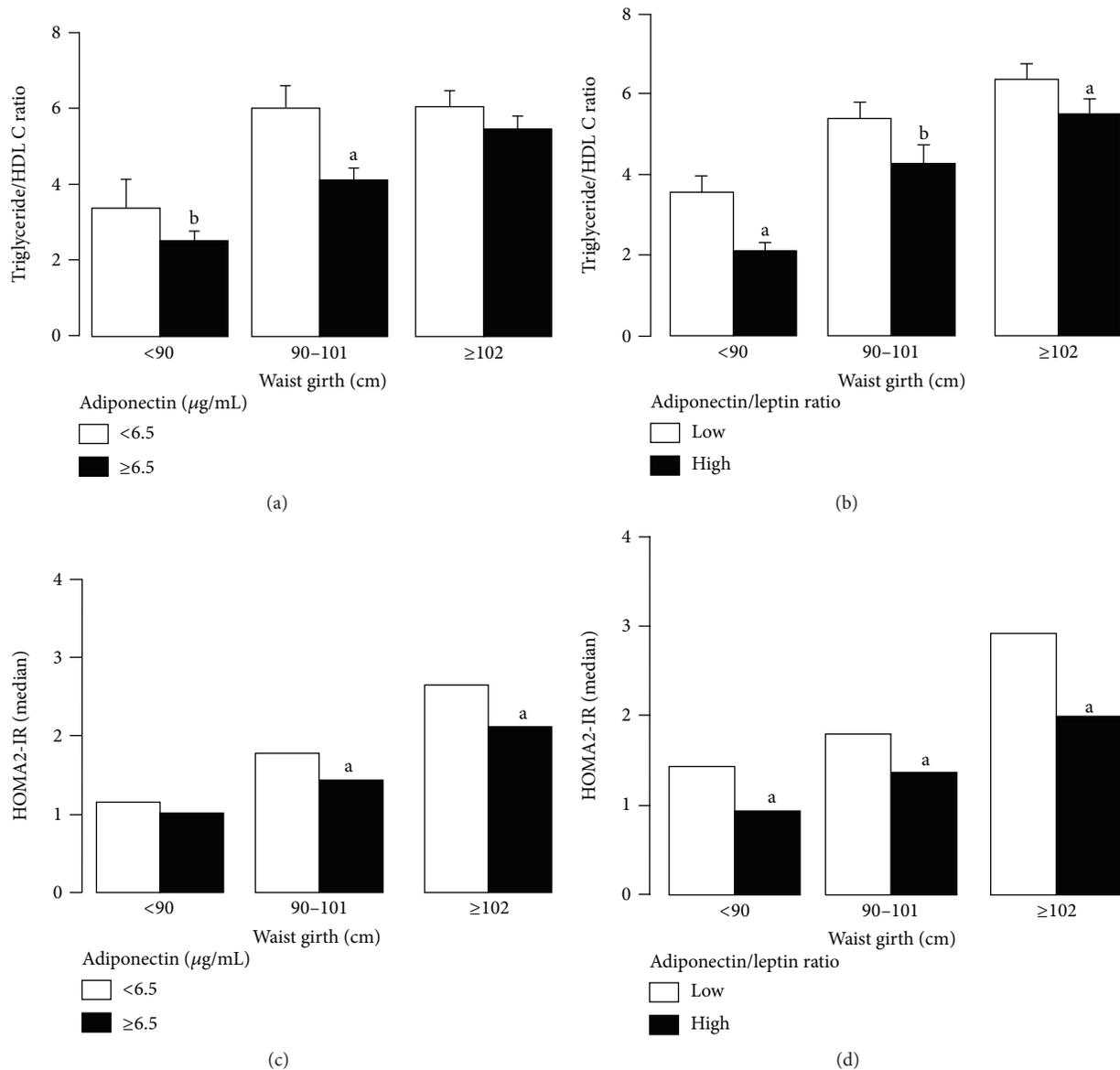


FIGURE 3: Ratio of triglyceride to HDL cholesterol (C) is lower in nonobese (^b $P < .05$) and overweight (^a $P < .02$) men who have a high level of plasma adiponectin (a). In contrast, the ratio of plasma triglyceride to HDL C is lower in nonobese, overweight, and obese subjects with a high ratio of adiponectin/leptin compared to those with a low ratio of adiponectin to leptin (b). HOMA2-IR levels are significantly higher (^a $P < .02$) in overweight and obese men with a high level of adiponectin compared with those with a low level of adiponectin (c). However, the HOMA2-IR levels are significantly lower in nonobese, overweight, and obese men who have a high ratio of adiponectin to leptin compared with those that have a low ratio (d).

of adiponectin/leptin compared to those with a low ratio of adiponectin/leptin (Figure 3(b)).

HOMA2-IR levels are significantly higher (^a $P < .02$) in overweight and obese men with a high level of adiponectin compared with those with a low level of adiponectin (Figure 3(a)). However, the HOMA2-IR levels are significantly lower in nonobese, overweight, and obese men who had a high ratio of adiponectin/leptin compared with those that have a low ratio (Figure 3(b)).

Systolic blood pressure in nonobese, overweight, and obese men was similar in those with low and those with high

level of adiponectin (Figure 4(a)) and in those with a high or low ratio of adiponectin/leptin (Figure 4(b)).

The ratio of adiponectin/leptin in subjects with and without risks for metabolic syndrome that excluded waist girth was also examined using the Kruskal-Wallis rank test (Figure 5). Subjects with ≥ 1 risk factors have a lower ratio than those without any risk factor. In addition, men with 2 risk factors have a significantly lower ratio than those with 1 risk, and those with ≥ 3 risks have significantly lower ratio than those with 1 or 2 risks.

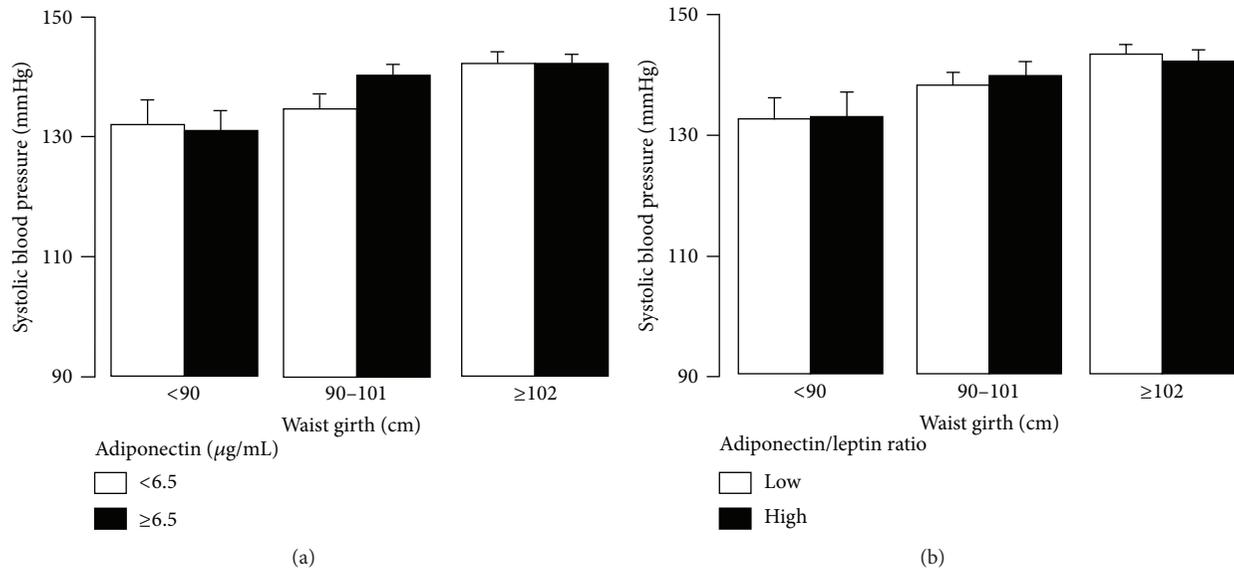


FIGURE 4: Systolic blood pressure in nonobese, overweight, and obese men was similar in those with low and those with high level of adiponectin (a). There were no significant differences in systolic blood pressure when the men were subgrouped according to the ratio of adiponectin/leptin (b).

6. Discussion

This study examined the relation of adiponectin levels alone or normalized by leptin to metabolic risk factors for cardiovascular disease including markers of atherogenic dyslipidemia (levels of plasma triglyceride and HDL cholesterol and ratios of plasma triglyceride/HDL cholesterol) and insulin resistance (levels of HOMA2-IR) in men. The key observations made in this study were that high levels of adiponectin were associated with lower plasma triglycerides, higher HDL cholesterol, reduced ratios of triglyceride/HDL cholesterol, and reduced HOMA2-IR compared to lower levels of adiponectin in men regardless of waist girth. Normalizing adiponectin levels by leptin enhanced the associations of adiponectin to the metabolic risk factors. That is, subjects with a high ratio of adiponectin/leptin had lower triglycerides and triglyceride/HDL cholesterol ratios and higher HDL cholesterol and insulin sensitivity than those with low ratios of adiponectin/leptin regardless of waist girth. Thus, the adiponectin/leptin ratio was a useful index for identification of overweight and obese subjects with lower susceptibility to metabolic risk compared to individuals with a higher susceptibility.

Both adiponectin and leptin have been implicated in the causation of dyslipidemia and insulin resistance. For example, leptin deficiency is associated with hypertriglyceridemia, low HDL C, and low insulin sensitivity in cases of acquired or congenital lipodystrophies [18]; leptin therapy reverses the metabolic dysfunction [19]. But in obese subjects, leptin does not have an effect [20]. Instead, leptin correlates with fat mass and it can be viewed as a biomarker of fat cell mass. Plasma levels of leptin generally reflect secretion rates by subcutaneous adipose tissue, principally by large adipocytes [9]. Omental fat also secretes leptin, but the subcutaneous fat

is thought to be a major source of leptin. This hormone is known to modulate energy homeostasis through its action on hypothalamic receptors where it inhibits appetite [21]. Mutations in the human leptin gene are associated with hypogonadism and morbid obesity [22], and mutations in the human leptin receptor gene causes obesity and pituitary dysfunction [23]. Leptin replacement in obese subjects with leptin deficiency reverses the metabolic consequences of the deficiency in the hypothalamus [24].

In the current study, the linear association between plasma levels of leptin and waist girth provided a rationale for using leptin as a surrogate of fat mass. Moreover, overweight and obese men showed considerable interindividual variation in leptin levels despite the high correlation with waist girth. The interindividual variation also suggested that leptin levels are probably a more specific measure of fat mass than waist girth. For these reasons, leptin was used to normalize levels of adiponectin.

In contrast to leptin levels, plasma levels of adiponectin were not strongly correlated with waist girth. Others have shown an inverse association of adiponectin with total body fat [25]. However, the current study population showed a marked heterogeneity in plasma adiponectin level and this proved to be instructive. At any waist girth category, it was clear that there were men with high and low adiponectin levels. Thus, two questions could be addressed readily: (1) are there differences in levels of metabolic risk factors between men with high and low adiponectin levels regardless of waist girth? (2) If adiponectin levels are “normalized” for leptin levels, are the differences in metabolic risk factors better defined by the ratio of adiponectin/leptin than adiponectin alone?

The levels of plasma triglyceride were lower in men with a high adiponectin than in those with a low adiponectin.

In contrast, HDL C, levels were higher in men who had high adiponectin compared with those with low adiponectin. Similarly, low ratios of plasma triglyceride/HDL C were lower in men with high adiponectin compared with those with low adiponectin. After normalizing adiponectin levels by leptin, the impact of the ratio of adiponectin/leptin on triglyceride, HDL C, and the ratio of triglyceride to HDL C was more apparent. The ratio segregated overweight and obese subjects into those with a relatively healthier metabolic profile and those with a higher-risk profile. Still the average plasma triglyceride levels of overweight and obese men with a high ratio of adiponectin/leptin were somewhat higher than the cut point of at-risk triglyceride (150 mg/dL). Perhaps a higher adiponectin/leptin ratio is needed to optimize triglyceride levels.

In contrast to leptin, adiponectin levels may be indicative of a protective effect of the adipokine on triglyceride metabolism even in the presence of excess body fat. Studies in animals suggest that adiponectin reduces levels of plasma triglyceride by increasing VLDL-triglyceride hydrolysis mediated by lipoprotein lipase [26]. Transgenic mice overexpressing adiponectin show a reduction in plasma triglycerides compared to wildtype [27–29] while adiponectin knockout mice have increased plasma triglycerides. These data suggest that adiponectin has a direct effect on triglyceride hydrolysis [30]. Other studies also suggest that adiponectin is an insulin sensitizer and it could exert a hypotriglyceridemic effect under such conditions. The association of adiponectin with HDL cholesterol may result from its hypotriglyceridemic effect or it could be the result of the effect of adiponectin on either apo A-I fractional catabolic rate [31, 32] or a direct effect of adiponectin on hepatic lipase [33, 34].

In the current study, high adiponectin levels also were generally associated with higher insulin sensitivity measured by HOMA2-IR than low adiponectin levels. This effect is supportive of the view that adiponectin is an insulin sensitizer [35, 36]. As such the adipokine can modulate the metabolism of triglycerides, HDL, and glucose. The exact mechanisms of the insulin sensitizing effect of adiponectin are not clearly understood. But in this study, it was clear that adiponectin and, more specifically, the ratio of adiponectin/leptin were a good indicator of insulin sensitivity in overweight and obese men.

The adiponectin/leptin ratio also was shown to decrease with the increasing number of metabolic risk factors for cardiovascular disease (Figure 5). This ratio may be useful to identify subjects susceptible to metabolic risk, and adiponectin/leptin ratios may reflect the functionality of adipose tissue. Accordingly, two metabolic phenotypes were identified in overweight and obese subjects in the current study. It has been suggested that subjects are heterogeneous in the prevalence of metabolic risk factors. Some nonobese subjects have “metabolic obesity” [37]. Other investigators have identified obese subjects with a low prevalence of metabolic alterations which they have designated as “metabolically healthy” or “unhealthy” [38–40]. In the current study, the individuals with the high ratio of adiponectin/leptin had less dyslipidemia and insulin resistance than those with the low ratio, but they were not free of other risk factors. The ratio,

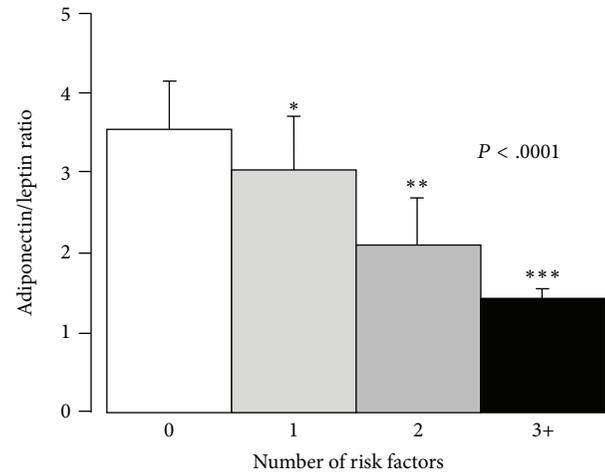


FIGURE 5: Plot of ratios of adiponectin/leptin versus number of risk factors. Subjects with ≥ 1 risk factors have a lower ratio than those without any risk factor. In addition, men with 2 risk factors have a significantly lower ratio than those with 1 risk, and those with ≥ 3 risks have significantly lower ratio than those with 1 or 2 risks.

however, suggested that adiponectin levels relative to leptin may be indices of susceptibility to metabolic risk.

7. Conclusion

The current study shows that overweight and obese individuals with a high ratio of adiponectin/leptin have lower levels of plasma triglyceride, triglyceride/HDL C ratios and higher insulin sensitivity than those with lower adiponectin/leptin ratios. It is very likely that adiponectin contributes to the regulation of plasma triglyceride levels. The data also suggests that interindividual variation in dyslipidemia and insulin sensitivity is associated with the ratio of the adipokines. The study provides supportive evidence for the contention that some overweight and obese subjects have a better adipokine profile than others and that metabolic heterogeneity among overweight and obese subjects may depend on the ability of adipocytes to maintain secretion of the adipokines as the cells become filled with triglyceride.

Conflict of Interests

The authors have no conflict of interests to report for the submitted paper.

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Review Article

The Role of Adipose Tissue in Insulin Resistance in Women of African Ancestry

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Women of African ancestry, particularly those living in industrialized countries, experience a disproportionately higher prevalence of type 2 diabetes (T2D) compared to their white counterparts. Similarly, obesity and insulin resistance, which are major risk factors for T2D, are greater in black compared to white women. The exact mechanisms underlying these phenomena are not known. This paper will focus on the role of adipose tissue biology. Firstly, the characteristic body fat distribution of women of African ancestry will be discussed, followed by the depot-specific associations with insulin resistance. Factors involved in adipose tissue biology and their relation to insulin sensitivity will then be explored, including the role of sex hormones, glucocorticoid metabolism, lipolysis and adipogenesis, and their consequent effects on adipose tissue hypoxia, oxidative stress, and inflammation. Finally the role of ectopic fat deposition will be discussed. The paper proposes directions for future research, in particular highlighting the need for longitudinal and/or intervention studies to better understand the mechanisms underlying the high prevalence of insulin resistance and T2D in women of African ancestry.

1. Introduction

The rising global burden of type 2 diabetes (T2D) is being borne primarily by low and middle income countries, including those in Africa, where there is recent evidence of a rising prevalence amongst urban black South Africans [1]. Further, people of African ancestry living in high income countries such as the United States of America (USA), United Kingdom, (UK) and Europe have a much higher prevalence of T2D than their respective countrymen and thereby also contribute to the increasing burden, albeit to a less extent [2]. The rising prevalence of T2D is being fuelled, in part, by the high rates of obesity in these communities. For example, 87% of all T2D in South Africa is attributable to elevated

body mass index (BMI) [3] and obesity rates are higher in African diaspora communities compared to residents of the respective high income countries, particularly amongst women [2].

Insulin resistance, a common accompaniment of obesity, is a significant risk factor for T2D. However, there are also ethnic differences in the level of insulin resistance, with black South African women and African American women being more insulin resistant than their white counterparts, even when matched for age and BMI [4–6]. Insulin resistance in women of African ancestry is associated with an appropriately greater insulin response to maintain normoglycemia [4, 7]. The resultant hyperinsulinemia is due to an increase in insulin secretion as well as reduced hepatic clearance [4, 7].

The latter has been proposed as a potential mechanism to protect the beta cell from exhaustion [8], although it does not protect women of African ancestry from T2D.

Although the exact mechanism is not known, there are numerous mechanisms that have been postulated to underlie the increased risk of T2D and insulin resistance in women of African ancestry. This paper will focus on the role of adipose tissue biology. The characteristic body fat distribution of women of African ancestry will be discussed first, followed by the depot-specific associations with insulin resistance, and then various biological factors within adipose tissue will be explored. As most studies have examined these topics by comparing black and white women, these comparative data are presented, and where available, the ethnic-specific associations with body fat distribution and insulin sensitivity are described.

2. Body Fat Distribution

Traditionally one of the major determinants of insulin resistance is the centralization of body fat [9], more specifically increased visceral adipose tissue (VAT) [10], while peripheral (gluteofemoral) subcutaneous adipose tissue (SAT) deposition has been shown to be “protective” against insulin resistance (in predominately white populations) [11]. Yet, many studies in South Africa and the USA have shown that at the same level of BMI or waist circumference, black women are more insulin resistant than their white counterparts, despite having less VAT [4–6]. The major contributor to abdominal fat in African women is SAT, largely superficial rather than deep SAT [12, 13], and is accompanied by greater peripheral (gluteo-femoral) fat [14]. Thus, irrespective of the size of the depot, there is evidence of ethnic differences in the association between regional fat distribution and insulin sensitivity. In white women, VAT is the most significant determinant of insulin sensitivity [10], whereas in black women, insulin sensitivity is more closely associated with abdominal SAT than VAT [5, 12, 13]. In black South African women, this association is stronger for the deep SAT than the superficial SAT depot [12]. Further, we showed for the first time that gluteal SAT is negatively correlated with insulin sensitivity in black, but not white women [14].

Several mechanisms have been proposed for the depot-specific associations between adipose tissue accumulation and insulin sensitivity. Compared to SAT, VAT is more lipolytic [15], has greater 11 β -hydroxysteroid dehydrogenase-1 (11HSD1) activity (which reactivates inactive cortisone to active cortisol) [16], and has reduced AMP-activated protein kinase (AMPK) levels [17] and a higher inflammatory profile [17, 18]. Notably, the products of VAT are released directly into the hepatic portal system. However, SAT, probably due to its sheer volume, accounts for ~80% of systemic circulating free fatty acids and produces large amounts of adipokines that have effects on the liver, skeletal muscle, and pancreas [19]. Abdominal deep SAT has been shown to be metabolically distinct to abdominal superficial SAT [20], displaying greater lipolytic activity [21], a lower intracellular pool of glucose transporter type 4 (GLUT4) [20], a higher inflammatory

profile [22], and a more saturated fatty acid profile [23]. Gluteo-femoral SAT differs from abdominal SAT in that it has higher lipoprotein lipase activity [24], is less lipolytic [25], has larger adipocytes [26], and has consequently been proposed as a “metabolic sink,” trapping excess free fatty acids (FFAs) and protecting against ectopic fat deposition [27]. Pinnick et al. [28] recently demonstrated that gluteo-femoral SAT has other insulin-sensitizing effects, as it expresses higher levels of stearoyl-CoA desaturase (SCD1), the key enzyme involved in the desaturation of palmitic acid (16:0) to palmitoleic acid (16:1n-7).

Although many factors have been purported to influence body fat distribution and the association with insulin sensitivity, sex hormones are considered one of the most relevant.

3. Sex Hormones

The centralization of fat distribution, in particular increasing VAT, in estrogen deficient states such as menopause and postovariectomy, is well known [29, 30]. However, there is scanty evidence linking alterations in estrogen levels or exposure, to the characteristic fat distribution seen in women of African ancestry. For example, Casazza et al. [31] showed that African American girls had higher estradiol levels and reached menarche at a younger age than white American girls. Menarche was associated with accelerated weight gain in African American girls only, but the distribution of fat between the upper and lower body did not differ by ethnicity. Further, studies examining ethnic differences in hormone levels in pre-, peri- and postmenopausal women are conflicting, with studies showing higher, similar, or lower levels of estrogen in African American compared to white American women [32–34]. Differences in body fat and its distribution, as well as age, menopause type, and health behaviors, are important factors explaining the discrepancies in sex hormone levels between women of different ethnicities. It is, however, relevant to bear in mind that the effects of estrogen are mediated by binding to its receptors alpha (ER α) and beta (ER β), with their relative ratios regulating the biological response [35]. Murine adipose tissue ER α and ER β knockout models have highlighted the role of ERs in regulating adipose tissue deposition and insulin sensitivity [36, 37]. However, there is a limited research in humans and their contribution to the ethnic differences in body fat distribution and insulin sensitivity that remains to be explored.

4. Adipose Glucocorticoid Metabolism

An additional factor that may underlie the ethnic-specific association between regional fat distribution and insulin resistance may be exposure to glucocorticoids. A case in point is the pathological condition of cortisol excess, Cushing’s syndrome, which is characterized by central obesity, glucose intolerance, and insulin resistance [38]. Studies examining changes in the activity of the hypothalamic-pituitary-adrenal (HPA) axis with obesity have found that circulating levels

of cortisol are normal, or even low [39]. However, glucocorticoid action on target tissues depends not only on the circulating concentrations, but also on the tissue sensitivity to glucocorticoids, which is dependent on 11HSD1, which converts inactive cortisone to active cortisol, and the glucocorticoid receptor- α (GR α). 11HSD1 is of potential pathogenic importance in body fat distribution and insulin resistance, since transgenic mice overexpressing 11HSD1 selectively in adipose tissue have increased VAT accumulation and insulin resistance [40]. In contrast, 11HSD1 knockout mice resist the development of insulin resistance, even on a high-fat diet [41]. In obese humans, 11HSD1 activity and mRNA levels are elevated in VAT [16] and abdominal SAT and associate with insulin resistance [16, 42].

In the absence of published studies that have compared glucocorticoid metabolism within adipose tissue depots in women of African ancestry, our preliminary data (Goedecke et al., unpublished) showed that abdominal and gluteal SAT 11HSD1 mRNA and activity increased with obesity, independent of ethnicity. In addition, in white but not black women, increased 11HSD1 activity in SAT was associated with increased VAT and reduced insulin sensitivity. On the other hand, abdominal and gluteal SAT GR α mRNA were down-regulated to a greater extent with obesity in black compared to white women. In black women only, reduced GR α mRNA in gluteal SAT correlated with reduced insulin sensitivity, possibly mediated via an associated increase in inflammatory gene expression and a decrease in peroxisomal proliferator-activated receptor- γ (PPAR- γ) and adiponectin expression. Intervention studies are required to gain a greater understanding of these findings.

5. Adipose Tissue Lipolysis

Many studies have examined ethnic differences in adipose tissue lipolysis in an attempt to explain the higher levels of insulin resistance in women of African ancestry compared to white women, matched for body fatness. The findings of these studies are, however, conflicting, showing lower, higher, and similar rates of lipolysis between obese black and white women, irrespective of whether the measurements were performed *in vivo* or *in vitro* (Table 1). Different techniques used to assess lipolysis, the age of the participants, the degree of glucose tolerance, and the ethnic differences in VAT and plasma insulin levels may explain these conflicting findings. Currently, however, there is no clear evidence to suggest that women of African ancestry have higher rates of lipolysis than their white counterparts, and that this might explain their disproportionately high prevalence of insulin resistance. Rather, differences in adipogenesis may be implicated.

6. Adipogenesis

Adipose tissue accumulation can occur either by hyperplasia (increased adipocyte number) or hypertrophy (increased adipocyte size), with the latter being more closely associated with insulin resistance [54]. Although the exact mechanism whereby hypertrophy exerts its effects on insulin signaling is

not known, a number of mechanisms have been proposed, including, amongst others, reduced adipogenic potential, increased cellular hypoxia, and increased oxidative stress.

We recently found an ethnic-specific association between obesity, adipogenesis, and insulin sensitivity [14]. Adipogenic and lipogenic genes were more highly expressed in the peripheral (gluteal) tissue of normal-weight black compared to normal-weight white women, but were down-regulated to a greater extent in obese black versus obese white women, with the latter being associated with reduced insulin sensitivity in black, but not white women [14]. These findings have been confirmed in two separate studies. In a sample of normal-weight black and white South African women, adipogenesis was greater in preadipocytes isolated from the mammary gland tissue of black than white women [55]. In contrast, the expression of genes involved in adipogenesis including PPAR γ , SCD1, and lipin-1 β was reduced in SAT of African American compared to white American women, who were on average obese [56]. Together, these results might suggest that reduced adipogenic potential may increase adipocyte hypertrophy leading to insulin resistance. Indeed, van Tienen et al. [57] showed the decreased expression of adipogenic genes in SAT preadipocytes isolated from T2D subjects compared to controls.

Earlier studies have shown larger adipocyte size in women of African ancestry compared to their white counterparts [58], and that gluteal (but not abdominal) adipocyte size correlated with insulin levels in postmenopausal African American women, but not white American women [26]. However, some researchers postulate that it is not the size of the adipocytes, but rather the distribution of cell size that may contribute to insulin resistance [59]. In preliminary results from our laboratory, black South African women had a larger median gluteal adipocyte size and a greater proportion of large gluteal adipocytes, while white women had a greater proportion of small cells (Keswell et al. unpublished). These data, together with our findings of negative correlation between gluteal fat mass and insulin sensitivity [14], suggest that central obesity may not be a good predictor of insulin resistance in women of African ancestry, but rather that gluteal adipocyte size may be a more sensitive indicator of insulin resistance.

7. Hypoxia

Hypertrophy of adipocytes, particularly when the expanding cell mass exceeds the compensatory vascular supply, results in hypoxia in adipose tissue, with a concomitant reduction in insulin sensitivity [60, 61]. Cellular adaptation to hypoxia is accomplished through the activation of an array of oxygen sensing transcription factors, including hypoxia inducible factor-1 (HIF-1) [62], which induces the expression of pro-angiogenic proteins such as vascular endothelial growth factor (VEGF) and pyruvate dehydrogenase kinase-1 (PDK-1) [63]. However, in obese adipose tissue, even though HIF-1 protein levels increase, VEGF does not increase [64] and is accompanied by reduced capillary density [60, 64]. Furthermore, plasminogen activator inhibitor-1 (PAI-1),

TABLE 1: Ethnic differences in adipose tissue lipolysis.

Participants	System	Ethnic differences in lipolysis	References
Nondiabetic, premenopausal, obese black and white US women	<i>In vivo</i>	Black < white for basal and insulin-suppressed systemic glycerol turnover, but not FFA turnover	Albu et al. [43]
Premenopausal, centrally obese black and white US women	<i>In vivo</i>	Black < white for basal and epinephrine-stimulated lipolysis	Racette et al. [44]
Morbidly obese black and white US women	<i>In vitro</i>	Black < white for basal lipolysis in VAT and SAT; black = white for isoproterenol-stimulated lipolysis, with and without insulin, in VAT and SAT	Barakat et al. [45]
Postmenopausal, overweight black and white US women	<i>In vitro</i>	Black > white for basal and insulin-inhibited lipolysis of abdominal SAT	Fried et al. [46]
Premenopausal, obese black and white South African (SA) women	<i>In vivo</i>	Black > white for basal and insulin-inhibited lipolysis of abdominal SAT	Van Der Merwe et al. [6]
Premenopausal, obese black and white SA women	<i>In vitro</i>	Black > white for insulin-inhibited lipolysis of abdominal SAT	Buthelezi et al. [47]
Premenopausal, normal-weight and obese black and white SA women	<i>In vivo</i>	Black = white for basal and insulin-suppressed lipolysis	Goedecke et al. [48]

a stimulator of angiogenesis, is upregulated during obesity in VAT and SAT depots [65]. Notably, Festa et al. [66] showed that the expression of PAI-1 was lower in women of African ancestry compared to white and Hispanic women. We, therefore, speculate that higher levels of hypoxia may increase the expression of HIF-1 in adipose tissue and result in higher levels of inflammation and oxidative stress in African women.

8. Oxidative Stress

Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and the cells antioxidant defense mechanisms that leads to the damage of proteins, lipids, and DNA. Although many studies have been performed in animal models to determine the association between oxidative stress and insulin resistance [67], human studies are limited. In healthy, nondiabetic women, increased protein carbonyls, a marker for oxidative stress, were associated with increased FFA and reduced insulin sensitivity in African Americans, but not European or Americans [68]. To our knowledge, no published studies have examined the association between oxidative stress within adipose tissue and insulin resistance, while a plethora of studies have examined the inflammatory profile of adipose tissue and its association with insulin resistance.

9. Adipose Tissue Inflammation

Adipose tissue of obese individuals and patients with T2D is characterized by increased expression and/or secretion of several proinflammatory cytokines (e.g., tumor necrosis factor- α (TNF- α), interleukin (IL)-18, IL-6), chemokines (e.g., C-C motif ligand (CCL 2)), macrophage markers (e.g., CD68 and CD14), and adipokines (leptin), and decreased expression of the insulin-sensitizing adipokine, adiponectin [69]. Studies in humans have shown that individuals with inflamed abdominal SAT (characterized by increased gene

expression of inflammatory proteins and presence of crown-like structures) are more hyperinsulinemic and insulin resistant compared to BMI-matched individuals without inflamed SAT [70].

A few studies have examined ethnic differences in the inflammatory profile of adipose tissue. In a sample of morbidly obese African American and white American women undergoing bariatric surgery, no ethnic differences in mRNA levels or *in vitro* release of IL-6, IL-8, and prostaglandin E₂ from VAT were reported [71]. In contrast, our group found that black South African women had a higher abdominal and gluteal SAT inflammatory gene expression (characterized by increased CCL2 and its receptor CCR2, CD68, TNF- α , colony stimulating factor- (CSF-1) and macrophage inhibitory factor (MIF)) than white women, independent of age, total adiposity, and VAT [72]. Despite being more insulin resistant and having a higher inflammatory profile, SAT inflammatory gene expression only accounted for 20% of the variance in insulin sensitivity in black SA women, compared to 56% in white SA women [72]. In contrast to our findings, Smith et al. [56] found that the mRNA levels of abdominal SAT CD68, leptin, and retinol binding protein-4 were similar between African American and white American women, matched for BMI and insulin sensitivity. This variation in findings may be attributable to the fact that adipocytokines act in an endocrine, paracrine, and autocrine manner, making it difficult to capture their true effects using traditional molecular biology techniques. Furthermore, differences in age, body fatness, and level of insulin sensitivity may explain the disparate findings between the studies.

With the exception of the adipocyte-derived proteins, adiponectin, and leptin, circulating levels of inflammatory proteins are largely derived from nonfat cells, including immune and endothelial cells within adipose tissue. As a result, ethnic differences in circulating levels of IL-6, IL-18, TNFR1, TNFR2, iCAM-1, VCAM-1, and adiponectin have largely been shown to be independent of the level of adiposity [73] (Table 2). However, in some instances, ethnic differences in circulating inflammatory proteins, including the acute

TABLE 2: Ethnic differences in the relationship between circulating inflammatory markers and insulin resistance.

Inflammatory marker	Ethnic difference	Moderating factors	Association with risk	References
hsCRP	Black > white	BMI and SES attenuate the difference between ethnic groups	Significant association with IR in both black and white populations	Festa et al. [49]
IL-6	Black > white	Minor attenuation after adjusting for body fatness	Significant association with IR in white women only	Hyatt et al. [50]
IL-18	Black > white/Hispanic	Not defined	Significant associations with IR glucose in both black and white populations	Zirlik et al. [51]
Adiponectin	White > black	Still significant after adjustment for age, BMI, or WHR	Significant associations with IR in white women only	Ferris et al. [52]; Hulver et al. [53]

hsCRP: high-sensitivity C-reactive protein; BMI: body mass index; SES: socioeconomic status; IL: interleukin; IR: insulin resistance; WHR: waist-hip ratio.

phase protein, C-reactive protein (CRP), and the adipokine, leptin, have been attributed to differences in adiposity [49]. Whether or not other confounding variables may explain these differences remains to be explored.

Studies comparing the association between inflammatory markers and risk factors for T2D between ethnic groups have shown inconsistent results for some, but not all inflammatory proteins. For example, higher circulating levels of the insulin-sensitizing and anti-inflammatory hormone adiponectin were associated with a more favorable metabolic profile (lower BMI, insulin levels, and HOMA-IR) in white, but not black women [52, 53]. Similarly, circulating levels of the pro-inflammatory cytokine IL-6 have been shown to correlate with insulin sensitivity in overweight white American women and not overweight African American women [50]. The heterogeneity in the associations between ethnic groups highlights the need for prospective studies investigating the role of circulating inflammatory markers for risk prediction in different populations.

10. Ectopic Fat Deposition

A lower capacity to store fat in the periphery and higher rates of lipolysis is associated with a redirection of excess lipids to nonadipose depots, notably the liver, muscle, heart, and pancreas, where they accumulate as ectopic fat, which has been linked to insulin resistance [27, 74]. Ectopic fat deposition in the liver and skeletal muscle, measured using ¹H magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI), was more tightly (negatively) correlated with systemic insulin sensitivity than was VAT volume [75]. However, this association does not hold true in all ethnic groups, as for the same level of body fat and/or insulin sensitivity, black women have less visceral and ectopic fat deposition than their white (or Hispanic) counterparts [5, 12, 76, 77] a phenomenon that is already present in black adolescent populations [78].

Hepatic steatosis (fatty liver) is associated with hepatic insulin resistance, characterized by decreased suppression of hepatic glucose production and lower insulin-stimulated liver glycogen synthesis. In a large multiethnic population-based study in the USA ($n = 2,287$), Browning et al. [77], using MRS, showed that the prevalence of hepatic steatosis varied with ethnicity, with African Americans having a significantly

lower prevalence than white and Hispanics (24% versus 33% and 45%, resp.), which could not be explained by ethnic differences in BMI, insulin sensitivity, or ethanol intake. Rather, that the ethnic differences in hepatic steatosis may be explained by the lower VAT in black compared to white women [76, 78]. This has also been found in a small study of obese premenopausal South Africa women matched for BMI and insulin sensitivity (Goedecke et al., unpublished). Thus, it appears that the relationship between hepatic steatosis and insulin sensitivity differs by ethnicity, with hepatic steatosis not being integral to the pathogenesis of insulin resistance in black women.

On the other hand, intermuscular adipose tissue (IMAT) stores (measured using computerized tomography (CT) and MRI), a depot similar in size to VAT, were greater in African American compared to white individuals [79, 80] and were inversely associated with insulin sensitivity [79, 80]. However, when MRS has been used to quantify both intramyocellular (IMCL) and extramyocellular lipid (EMCL) content, only EMCL [81], and not IMCL [56, 81], was increased in African American and black South Africans (Goedecke et al., unpublished). Further, IMCL was associated with insulin sensitivity in white, but not black women and girls [81].

Increased skeletal muscle fat accumulation may be attributed to increased fatty acid availability and uptake and/or reduced fatty acid oxidation. Compared to their white counterparts, African American women have higher postabsorptive skeletal muscle lipoprotein lipase activity and are metabolically inflexible [82]. Further, *in vitro* studies have shown lower rates of skeletal muscle fatty acid oxidation [83], as well as reduced mitochondrial function in black compared to white premenopausal African American women [84]. Ethnic differences in the oxidative potential of the muscle may be related to muscle fiber composition, as African American women have been shown to have less type I oxidative fibers and more type IIb glycolytic fibers than their white counterparts [85]. The accumulation of by-products of fatty acid oxidation, such as diacylglycerol (DAG), ceramides, and long chain acyl-CoA, rather than IMAT alone, is associated with the development and progression of insulin resistance [86]. In addition, fat tissue releases many adipokines, including proinflammatory cytokines, as well as ROS that interfere with the insulin signaling pathway, thus impairing skeletal muscle

glucose uptake. Further research is required to determine whether these by-products and adipokines explain the ethnic differences in insulin sensitivity.

11. Conclusion

This paper highlights the complexity of adipose tissue biology in the pathogenesis of insulin resistance and T2D in women. We have presented evidence that body fat distribution and adipose tissue biology and their association with insulin resistance differ by ethnicity. In summary, the research to date has shown that despite women of African ancestry being more insulin resistant than their white counterparts, they have less VAT and hepatic steatosis and more peripheral SAT. The larger SAT adipocyte size in women of African ancestry is associated with a reduced adipogenic capacity and a higher expression of inflammatory genes compared to their white counterparts. Given that most of the studies reviewed are cross-sectional in nature, causality cannot be inferred. Further, it is not known whether the ethnic differences in body composition can be explained by differences in SAT ER or GR expression, or whether adipose tissue hypertrophy in women of African ancestry is associated with increased hypoxia and/or oxidative stress in SAT and consequently insulin resistance. Longitudinal and interventional studies are critical for a better understanding of the mechanisms underlying the disproportionately high prevalence of insulin resistance and T2D in women of African ancestry.

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